

Side chain and backbone structure-dependent subcellular localization and toxicity of conjugated polymer nanoparticles

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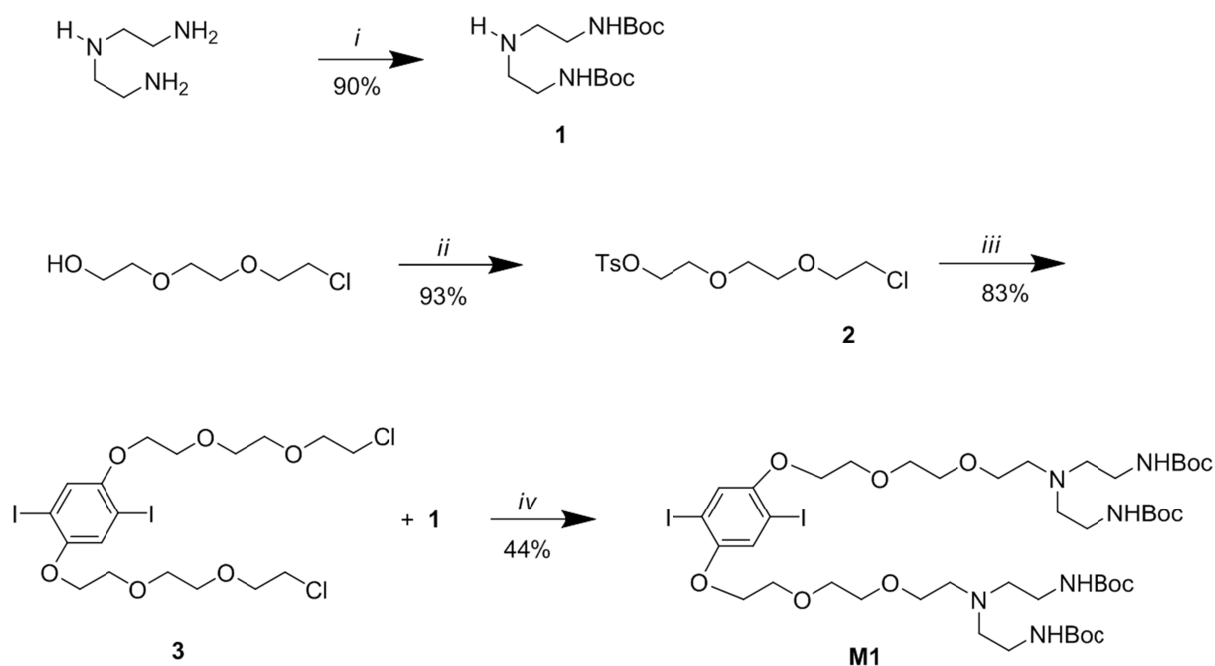
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Experimental

General Data. Chemicals, including solvents, were purchased from Fisher Scientific and used as received. Deuterated solvents were purchased from Cambridge Isotope Laboratories (Cambridge, MA). UV-vis spectra were recorded using a Varian Cary 50 Bio spectrophotometer. Fluorescence spectra were obtained using a FluoroLog-3 Spectrofluorometer (Jobin Yvon/Horiba). 9,10-diphenylanthracene (QY = 1.0) in cyclohexane was used as a fluorescence standard for QY determination. Hydrodynamic radii and Zeta potentials were determined by the dynamic light scattering technique using a Zetasizer nano-ZS (Zen 3600, Malvern Instruments Ltd., Worcestershire, United Kingdom). Disposable capillary cell (DTS1061, Malvern Instruments Ltd., Worcestershire, United Kingdom) used for zeta potential measurements. Low volume disposable cuvettes (ZEN0040, Malvern Instruments Ltd., Worcestershire, United Kingdom) used for DLS analysis. The average molecular weight (M_n) and polydispersity index ($PDI = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel 5 μ m MIXED-D columns and SPD-20A ultraviolet-visible (UV-vis) detector. Nuclear magnetic resonance (NMR) spectra were recorded on a 400 MHz and 600 MHz Avance Bruker NMR spectrometer. The 600 MHz NMR spectrometer is equipped with a gradient system capable of producing magnetic field pulse gradients in the z-direction of about 50 G cm^{-1} and allowing for water peak suppression ($\delta = 4.8$ ppm in D_2O). Experiments were carried out using a 5 mm BBI probe and the temperature was 298K. The mixing time was 300ms water suppression experiments. Chemical shifts were reported in parts per million (ppm) for 1H -NMR on the δ scale based on the middle peak ($\delta = 2.50$ ppm) of the dimethyl sulfoxide (DMSO)- d_6 solvent as an internal standard, or 4.8ppm for deuterium hydroxide (D_2O) experiments. Dialysis and solvent exchange of CPNs were conducted using an Ultrafiltration Stirred Cell (Millipore, Billerica, MA, USA) with membrane filters [Ultracel ultrafiltration disc, molecular weight cutoff (MWCO): 30 kDa]. Tables and graphs were plotted using Origin 8 software (OriginLab, Northampton, MA, USA).

Monomer Synthesis.

Monomer 1 (**M1**) was synthesized according to Scheme 1. tert-butyl 2,2'-azanediylbis(ethane-2,1-diyl)dicarbamate **1** and 2-(2-(2-chloroethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **2** were synthesized according to literature procedures.^{1, 2} Condensation of **2** and 2,5-diiodohydroquinone in the presence of K₂CO₃ in 2-butanone resulted in **3**. Reaction of **1** with **3** using K₂CO₃ base in acetonitrile resulted in the formation of **M1**. **3** and **M1** were fully characterized by NMR and mass spectroscopy.

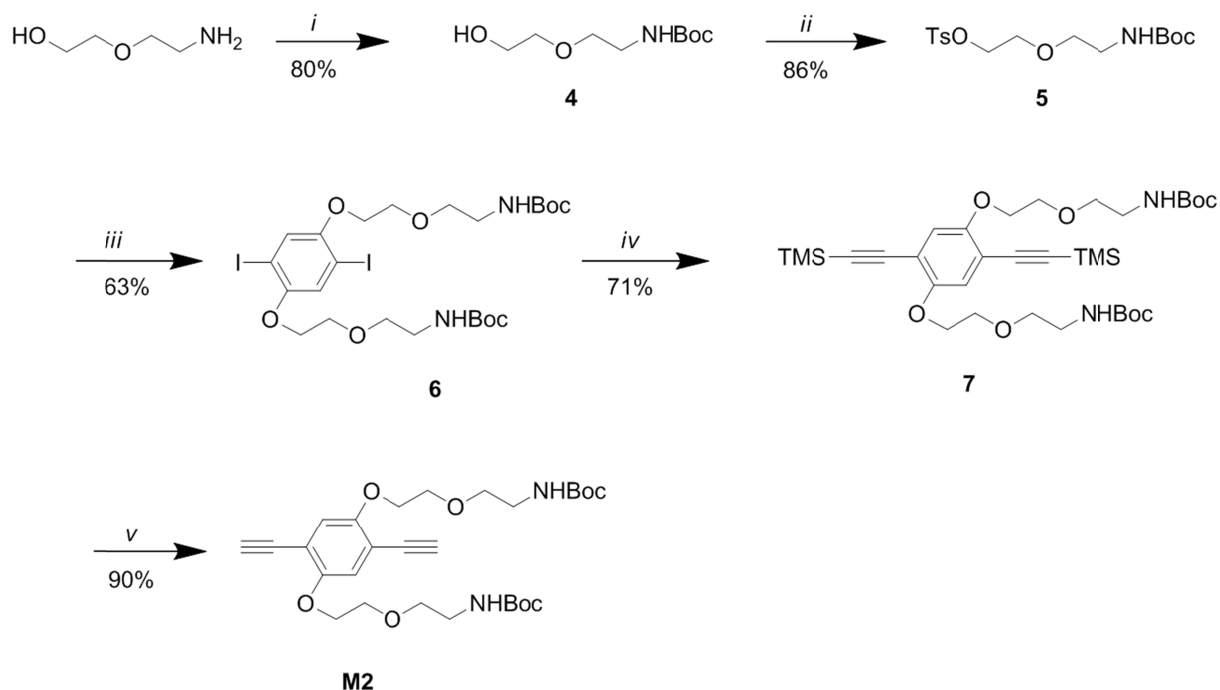


Scheme 1: Synthetic route towards monomer **M1**. Reagents and conditions: i) BocON, TEA, THF, 0°C, 3h; ii) TsCl, TEA, MC, room temp., overnight; iii) 2,5-diiodohydroquinone, K₂CO₃, 2-butanone, reflux, 1 day; iv) K₂CO₃, MeCN, Reflux, 3 days.

1,4-bis(2-(2-(2-chloroethoxy)ethoxy)ethoxy)-2,5-diiodobenzene (3): A suspension of compound **2** (5.89 mmol; 1.9 g), 2,5-diiodohydroquinone (2.76 mmol; 1 g) and potassium carbonate (8.28 mmol; 1.14 g) in 40 mL of 2-butanone and was refluxed for 1 day. The mixture was concentrated in vacuo and diluted with 30 mL of ethyl acetate. The solution was washed with brine (10 mL x3), dried over Na₂SO₄, and evaporated in vacuo. The crude product was purified by column chromatography (silica gel, ethyl acetate/ hexane(1:5, v/v) and Yield: 1.5 g (83%). ¹H-NMR(400 MHz) : δ = 7.22 (s, 1H, Ar-H), 4.09 (t, 2H, Ar-O-CH₂, J = 6) , 3.87 (t, 2H, CH₂O, J = 6), 3.82-3.74 (m, 4H, OCH₂CH₂), 3.69 (t, 2H, CH₂Cl), 3.61 (t, 2H, OCH₂, J = 6). ¹³C-NMR(400 MHz) : δ = 153.13, 123.52, 86.46, 72.01, 71.43, 71.15, 70.80, 70.33, 69.70, 42.83. HRMS (FTICR): Calcd. for C₁₈H₂₆Cl₂I₂O₆ [M+Na]⁺ = 679.9534; found [M+Na]⁺: 679.9529.

tert-butyl 2,2',2'',2'''-[2,2'-(2,2'-[2,2'-(2,5-diiodo-1,4-phenylene)bis(oxy)bis(ethane-2,1-diyl)]-bis-(oxy)bis(ethane-2,1-diyl)]bis(oxy)bis(ethane-2,1-diyl)]bis(azanetriyl)tetrakis(ethane-2,1-diyl)-tetracarbamate (M1): A suspension of compound 1 (1.51 mmol; 1 g), compound 3 (3.17 mmol; 0.96 g) and potassium carbonate (3.171 mmol; 0.28 g) in 40 mL of MeCN was refluxed for 3 days. The mixture was concentrated in vacuo and diluted with dichloromethane 30 mL. The solution was washed with brine (10 mL x3), dried over Na₂SO₄, and evaporated in vacuo. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane (1:1, v/v). Yield: 0.4g (44%) R_f=0.47 (3:1 ethyl acetate/hexane). ¹H-NMR(400 MHz) : δ = 7.25 (s, 1H, NH-COO), 7.23 (s, 1H, Ar-H), 4.10 (t, 2H, Ar-O-CH₂, J = 6) , 3.90 (t, 2H, CH₂O, J = 6), 3.82 (t, 2H, OCH₂, J = 6), 3.68 (t, 2H, OCH₂, J = 6), 3.52(br,2H, OCH₂), 3.14 (br, 4H, CH₂NHBoc, J = 6), 2.68 (br,2H,CH₂NH), 2.62 (br,4H, NHCH₂) 1.45 (s, 18H, OC(CH₃)₃); ¹³CNMR(400 MHz): δ = 156.9, 144.3, 121.1, 83.7, 77.2, 70.6, 70.4, 70.3, 70.1, 59.1, 54.2, 53.7, 51.2, 50.7, 38.9, 27.3. HRMS (FTICR): Calcd. for C₄₆H₈₂I₂N₆O₁₄ [M+H]⁺: 1197.4051; found [M+H]⁺: 1197.4032.

Monomer 2 (**M2**) was synthesized according to Scheme 2. tert-butyl 2-(2-hydroxyethoxy)ethylcarbamate **4**, 2-(2-(tert-butoxycarbonylamino)ethoxy)ethyl 4-methylbenzenesulfonate **5**, and tert-butyl 2,2'-[2,2'-(2,5-diiodo-1,4-phenylene)bis(oxy)bis(ethane-2,1-diyl)]bis(oxy)bis(ethane-2,1-diyl)dicarbamate **6** were synthesized according to literature procedures.³⁻⁵ Coupling of **6** with trimethylsilylacetylene using Sonogashira coupling yielded compound **7**, which was trimethylsilyl-deprotected to yield **M2**. Compounds **7** and **M2** were fully characterized by NMR and mass spectroscopy.

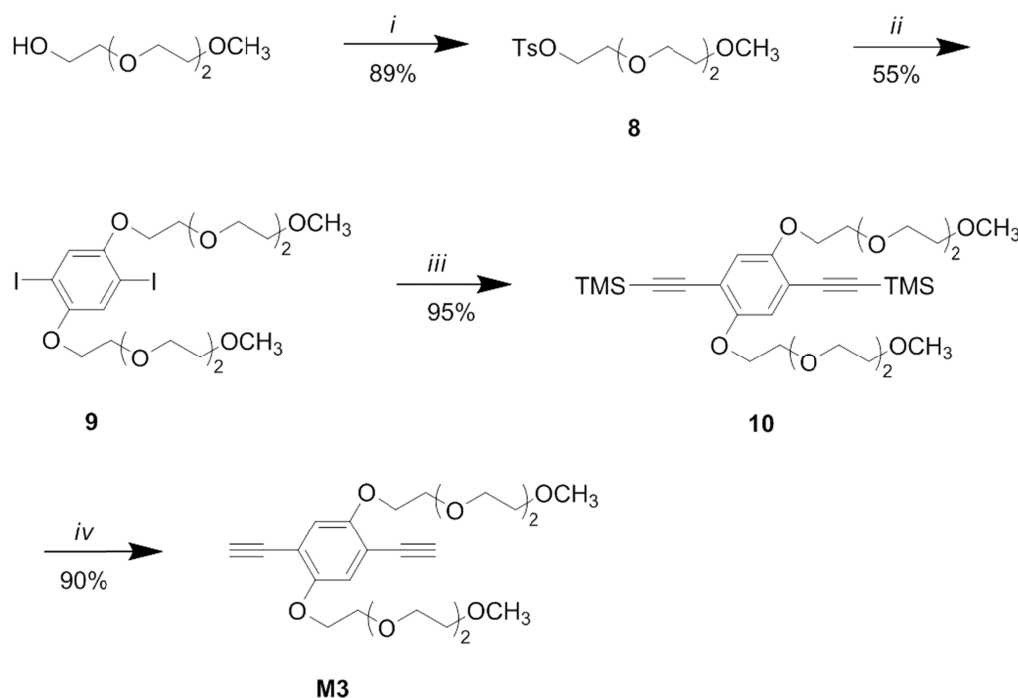


Scheme 2: Synthetic route towards monomer **M2**. Reagents and conditions: i) Boc_2O , MC, room temp., overnight; ii) TsCl, TEA, MC, room temp., overnight; iii) 2,5-diiodo-1,4-benzoquinone, K_2CO_3 , DMF, 80°C , 1 day; iv) Trimethylsilylacetylene, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, CuI, diisopropylamine, THF, 60°C , 12 h; v) K_2CO_3 , MeOH, room temp., overnight.

tert-butyl 2,2'-(2,2'-(2,5-bis[(trimethylsilyl)ethynyl]-1,4-phenylene)bis(oxy)bis(ethane-2,1-diyl))bis-(oxy)bis(ethane-2,1-diyl)dicarbamate (7): Compound 6 (2.03 mmol, 1.5 g) was added to a Schlenk flask fitted with a stir bar, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.203 mmol, 146 mg) and CuI (0.100 mmol, 20 mg). A solution of THF (20 mL) and diisopropylamine (10 mL) was then added to the reaction mixture. Subsequently, trimethylsilylacetylene (5.10 mmol, 0.7 mL) was added to the reaction mixture. The reaction was allowed to proceed for 12 h at 60°C , cooled to room temperature and the solvent was evaporated. The suspension was re-dissolved with methylenechloride, washed with saturated ammonium chloride (2×30 mL), and then dried over magnesium sulfate. The solvent was evaporated to produce dark brown oil, which was purified by column chromatography (silica gel, ethyl acetate/ hexane (4:1, v/v). Yield : 0.94g (71 %). $^1\text{H-NMR}$ (400 MHz) : δ = 7.23 (s, 1H, Ar-H), 5.0 (s, 1H, $\text{CH}_2\text{-NH}$), 4.10 (t, 2H, Ar- OCH_2 , J = 6), 3.81 (t, 2H, OCH_2 , J = 6), 3.83 (t, 2H, OCH_2 , J = 6), 3.65 (s, 1H, ArCCH), 3.36 (q, 2H, CH_2NH , J = 6), 1.43 (s, 10H, $\text{OC}(\text{CH}_3)_3$), 0.05 (s, 9H, TMS). $^{13}\text{C-NMR}$ (400 MHz) : δ = 171.1, 156.0, 154.0, 118.1, 114.5, 100.8, 79.1, 77.5, 71.9, 70.6, 69.6, 68.0, 60.4, 53.5, 41.3, 28.4, 21.0, 19.2, 14.2. HRMS (FTICR): Calcd. for $\text{C}_{34}\text{H}_{56}\text{N}_2\text{O}_8\text{Si}_2$ $[\text{M}+\text{H}]^+$: 677.3648; found $[\text{M}+\text{H}]^+$: 677.3644.

tert-butyl 2,2'-[2,2'-(2,5-diethynyl-1,4-phenylene)bis(oxy)bis(ethane-2,1-diyl)]bis(oxy)bis(ethane-2,1-diyl)dicarbamate (M2): A 20 mL vial was charged with compound **8** (1.42mmol, 0.400 g), Potassium carbonate (0.70mmol, 0.100 g) 15 mL of MeOH and left to stir overnight at room temperature. The resulting mixture was gravity filtered, the solvent was evaporated and the reaction mixture was purified by column chromatography (silica gel, ethyl acetate). Yield : 0.34g (90 %). $^1\text{H-NMR}$ (400 MHz) : δ = 7.00 (s, 1H, Ar-H), 5.0 (s, 1H, CH₂-NH), 4.15 (t, 2H, Ar-OCH₂, J = 6), 3.81 (t, 2H, OCH₂, J = 6), 3.65 (t, 2H, OCH₂, J = 6), 3.39 (s, 1H, ArCCH), 3.36 (q, 2H, CH₂NH, J = 6), 1.56 (s, 10H, OC(CH₃)₃); $^{13}\text{C-NMR}$ (400 MHz) : δ = 156.7, 154.1, 118.3, 113.7, 83.0, 79.5, 71.9, 70.6, 69.3, 69.25, 40.7, 28.4. HRMS (FTICR): Calcd. for C₂₈H₄₀N₂O₈ [M+H]⁺: 533.2857; found [M+H]⁺: 533.2849.

Monomer **3 (M3)** was synthesized according to Scheme 3. 2-(2-methoxyethoxy)ethyl 4-methylbenzenesulfonate **8**, 1,4-diiodo-2,5-bis(2-(2-methoxyethoxy)ethoxy)benzene **9**, (2,5-bis(2-(2-methoxyethoxy)ethoxy)-1,4-phenylene)bis(ethyne-2,1-diyl)bis(trimethylsilane) **10**, and 1,4-diethynyl-2,5-bis(2-(2-methoxyethoxy)ethoxy)benzene **M3** were synthesized according to literature procedures.⁶

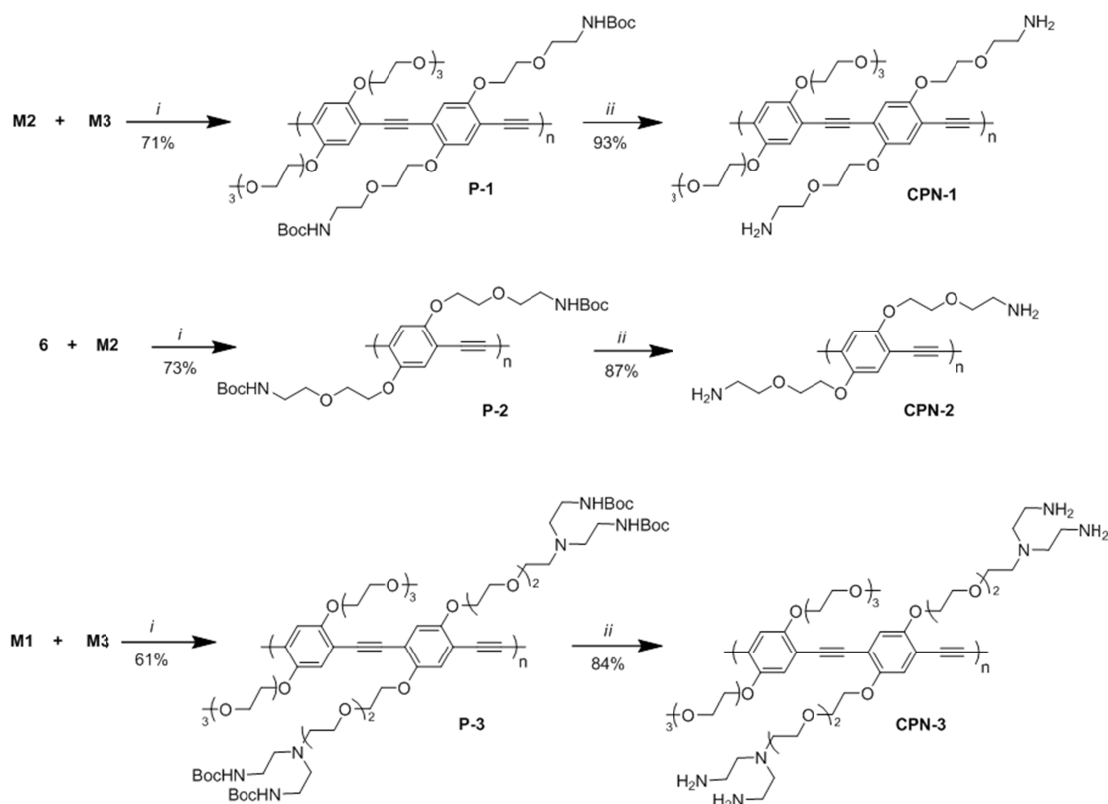


Scheme 3: Synthetic route towards monomer **M2**. Reagents and conditions: i) TsCl, TEA, Me, room temp., overnight; ii) 2,5-diiodohydroquinone, K₂CO₃, DMF, 80°C, overnight; iii) Trimethylsilylacetylene, Pd(PPh₃)₂Cl₂, CuI, diisopropylamine, THF, 60°C, 12 h; iv) 1M KOH(aq), THF, MeOH, room temp., overnight.

Polymer Synthesis.

General Procedure. A Schlenk flask was charged with aryl halide monomer (1.0 equiv) and diactylene monomer (1.0 equiv), along with Pd[(PPh₃)₂Cl₂] (0.2 equiv) and CuI (0.95 equiv). The Schlenk flask was evacuated and filled with N₂ three times. A solution of anhydrous dimethylformamide (DMF) (4 mL) and freshly distilled trimethylamine (1 mL) was degassed, and 1 mL of the mixed solution was transferred to the Schlenk flask using a cannular needle. The reaction was heated at 70 °C for 14 h. The solution was then cooled to room temperature and transferred dropwise to cold ethyl ether, resulting in precipitation. After centrifugation (2 min, 4000 rpm), the supernatant was decanted, and the precipitate was redissolved in DMF (1 mL) for further purification. See Scheme 4.

CPN Formation. Boc-deprotection of the polymer was carried out by mixing a solution of polymer in DMSO-d₆ with acetic acid (2 mL) and trifluoroacetic acid (1 mL) and allowed to stir at room temperature for 5 days. The mixture was then added to acetic acid (20 mL), allowed to stir overnight, and centrifuged, and supernatant was added dropwise (2 drops/s) to 500 mL water (18 Ω) while stirring. Using a solvent-resistant stir cell fitted with a 30 kDa-MWCO membrane, the solution was concentrated to approximately 10 mL, and dialyzed against 1 L of water. The resulting solution was further dialyzed in a 10 kDa membrane for 3 days. The solution was subsequently filtered through a Teflon (PTFE) syringe filter (0.45 μm) and used for further analysis. Deprotection was confirmed with ¹H-NMR. See Scheme 4.



Scheme 3: Synthetic route towards **CPN-1**, **CPN-2** and **CPN-3**. Reagents and conditions: i) Pd(PPh₃)₂Cl₂, CuI, TEA, DMF, 70°C, 14 h; ii) TFA, AcOH, DMF, room temp., 5 days.

P1. Using the general procedure described above, the polymerization of monomer **9** (32 mg, 0.044 mmol) and monomer **13** (20mg, 0.044mmol) in the presence of Pd[(PPh₃)₂Cl₂] (3 mg, 0.0044 mmol) and CuI (0.2 mg, 0.0022 mmol) yielded PPE polymer **P-1** (32mg, 71%). Molecular weight of boc-protected polymer was obtained and characterized. Resulting mixture was purified by precipitation in cold ethyl ether (x3). An aliquot was redissolved in THF and molecular weight obtained and photophysical properties characterized. The rest of the material was dissolved in DMSO-d₆ and characterized using ¹H-NMR. CPN formation was then carried out as described in general procedure to yield **CPN-1**.

P1: GPC: M_w = 24.43 kDa; M_n = 16.4 kDa; PDI = 1.49. UV λ_{max} = 427 nm; fluo λ_{max}(400nm ex) = 471 nm; QY = 41% in DMF. ¹H-NMR(400 MHz): δ = 7.35 (br, 1H, Ar-H), 6.87 (br, 0.5H, NHCOO), 4.08 (br, 2H, Ar-OCH₂), 3.91 (br, 2H CH₂O), 3.82 (br, 2H, OCH₂), 3.58 (br, 2H, CH₂O), 3.48 (br, 2H, OCH₂), 3.34 (br, 2H, CH₂O), 3.27 (br, 2H, OCH₂), 2.72 (br, 3H, OCH₃), 1.47 (s, 8H, OC(CH₃)₃). FT-IR (Neat): ν = 3372 (br), 2982 (w), 2935 (m), 2875 (s), 1710 (vs; C=O), 1513 (vs), 1461 (w), 1425 (m), 1425 (m), 1393 (m), 1363 (s), 1276 (s), 1219 (s), 1172 (m), 1120 (m), 1060 (m), 1033 (m).

CPN-1: UV λ_{\max} = 433 nm; fluo λ_{\max} (400nm ex) =496 nm; QY = 3% in water. $^1\text{H-NMR}$ (600 MHz) : δ = 7.36 (br, 1H, Ar-H), 4.3-3.0 (br, 23H, $-\text{OCH}_2\text{-CH}_2-$ chains), 1.30 (s, 0.21H). FT-IR (Neat): ν = 3374 (br), 2982 (w), 2935 (m), 2875 (s), 1674 (br), 1610 (w), 1500 (m), 1461 (w), 1423 (w), 1390 (w), 1360 (m), 1279 (w), 1250 (w), 1213 (br), 1104 (br), 1059 (br), 1035 (m).

P2. Using the general procedure described above, the polymerization of **6** (30 mg, 0.056 mmol) and monomer **M2** (41mg, 0.056mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (4 mg, 0.0056 mmol) and CuI (0.3 mg, 0.0028 mmol) yielded PPE polymer **P2** (44mg, 73%). Resulting mixture was purified by precipitation in cold ethyl ether (x3). An aliquot was redissolved in THF and molecular weight obtained and photophysical properties characterized. The rest of the material was dissolved in DMSO-d_6 and characterized using $^1\text{H-NMR}$. CPN formation was then carried out as described in general procedure to yield **CPN-2**

P2: GPC: M_w =16.9 kDa; M_n = 11.8 kDa; PDI = 1.43. UV λ_{\max} = 432 nm; fluo λ_{\max} (400nm ex) =475 nm; QY = 37% in DMF. $^1\text{H-NMR}$ (400 MHz, DMSO-d_6 , δ): 7.16 (s, 1H, Ar-H), 6.72 (s, 0.2H, NH-Boc), 4.21 (br, 2H, Ar- OCH_2), 3.80 (br, 2H, CH_2O), 3.66 (br, 2H, OCH_2), 3.52 (br, 2H, CH_2NH), 1.37 (s, 8H, $\text{C}(\text{CH}_3)_3$). FT-IR (Neat): ν = 3366 (br), 2933 (w), 2865 (s), 1704 (vs; C=O), 1507 (vs), 1457 (w), 1423 (w), 1365 (s), 1277 (w), 1247 (w), 1219 (s), 1172 (w), 1108 (s), 1057 (s), 1026 (w), 945 (s), 860 cm^{-1} (m).

CPN-2: UV λ_{\max} = 427 nm; fluo λ_{\max} (400nm ex) =492 nm; QY = 2% in water. $^1\text{H-NMR}$ (600 MHz, D_2O , δ): 7.03 (br, 1H, Ar-H), 4.24-2.76 (br, 10.74H, $\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2$), 1.36 (s, 0.46H). FT-IR (Neat): ν = 3417 (br), 2925 (w), 2865 (s), 1647 (br), 1495 (w), 1462 (w), 1418 (w), 1357 (w), 1283 (w), 1205 (s), 1094 (s), 1040 (w), 936 (w), 841 (w) cm^{-1} .

P3. Using the general procedure described above, the polymerization of monomer **4** (65 mg, 0.054 mmol) and monomer **13** (32mg, 0.054mmol) in the presence of $\text{Pd}[(\text{PPh}_3)_2\text{Cl}_2]$ (8 mg, 0.0054 mmol) and CuI (0.4 mg, 0.0022 mmol) yielded PPE polymer **P3** (45mg, 61%). Resulting mixture was purified by precipitation in cold ether (x3). An aliquot was redissolved in THF and molecular weight obtained and photophysical properties characterized. The rest of the material was dissolved in DMSO-d_6 and characterized using $^1\text{H-NMR}$. CPN formation was then carried out as described in general procedure to yield **CPN-3**.

P3: GPC: M_w =17.5 kDa; M_n = 10.7 kDa; PDI = 1.64. UV λ_{\max} = 420 nm; fluo λ_{\max} (400nm ex) =469 nm; QY = 35% in DMF. $^1\text{H-NMR}$ (400 MHz) : δ = 7.70-6.95 (br, 1H, Ar-H), 4.0-3.24 (br, 42H, OCH_2CH_2), 2.30-1.94 (br, 6H, $\text{NCH}_2\text{CH}_2\text{NH}$), 1.30 (s, 10H, $\text{OC}(\text{CH}_3)_3$). FT-IR (Neat): ν = 3357 (br), 2909 (br), 1656 (vs; C=O), 1500 (w), 1441 (w), 1421 (w), 1391 (w), 1367 (m), 1340 (w), 1316 (w), 1280 (w), 1250 (m), 1228 (w), 1208 (w), 1162 (m), 1103 (m), 1060 (br), 1034 (br).

CPN-3: UV λ_{\max} = 420 nm; fluo λ_{\max} (400nm ex) =496 nm; QY = 2% in water. $^1\text{H-NMR}$ (600 MHz) : δ = 7.70-6.45 (br, 1H, Ar-H), 4.3-2.5 (br, 28H, $\text{OCH}_2\text{-CH}_2-$ chains), 2.17 (br, 4H, $\text{NCH}_2\text{CH}_2\text{NH}$) FT-IR

(Neat): $\nu = 3369$ (br), 2982 (w), 2936 (m), 2875 (br), 1685 (br), 1631 (br), 1500 (br), 1460 (w), 1426 (s), 1361 (br), 1358 (br), 1279 (m), 1249 (w), 1203 (vs), 1181 (w), 1103 (br), 1047 (br).

Polymer P4. Polymer synthesis, deprotection, CPN fabrication, and characterization is described elsewhere.⁷

Supporting Data.

Polymer Deprotection. Confirmed using $^1\text{H-NMR}$ data by looking at characteristic Boc proton peak (1.36ppm) and $\sim 1700\text{ cm}^{-1}$ infrared band for the carbonate (C=O) group. IR samples were prepared by lyophilization to prepare neat sample. IR scans from 4000 to 700 cm^{-1} are presented below, depicting absence of boc-carbonate group and confirming deprotection.

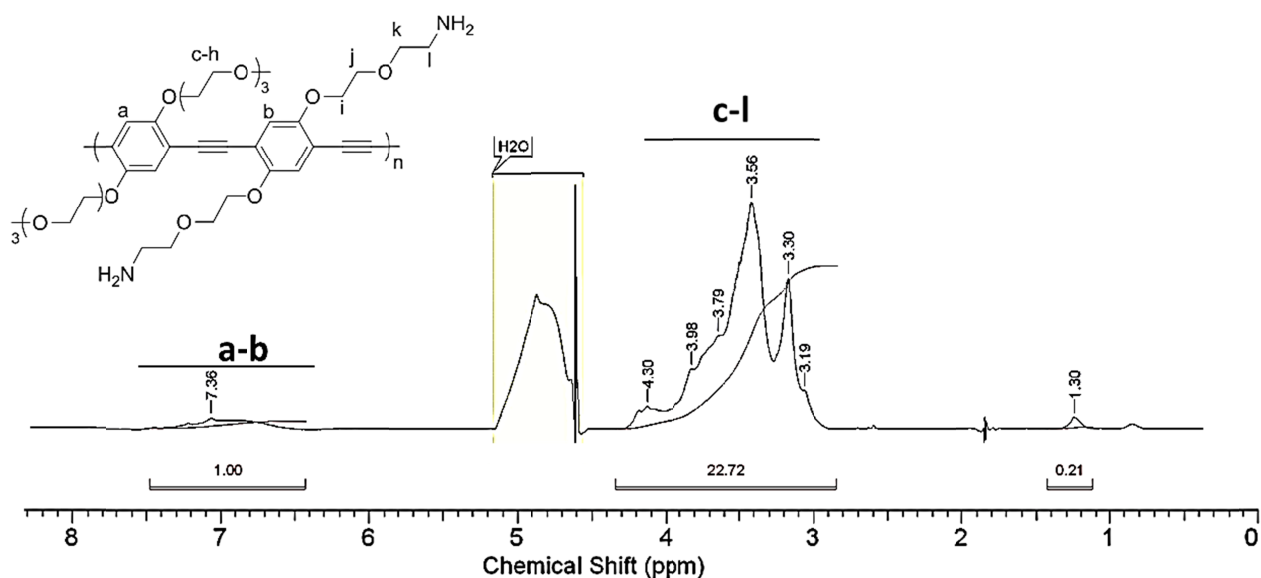


Fig S-1. $^1\text{H-NMR}$ for polymer CPN-1 in D_2O

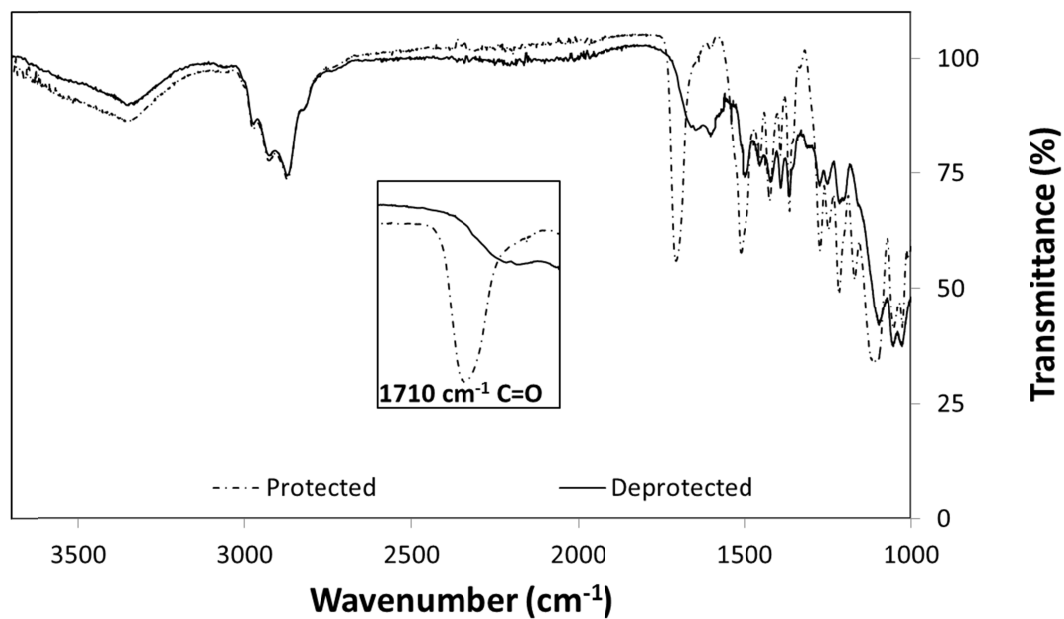


Fig S-2. FT-IR for polymer P1 (Neat)

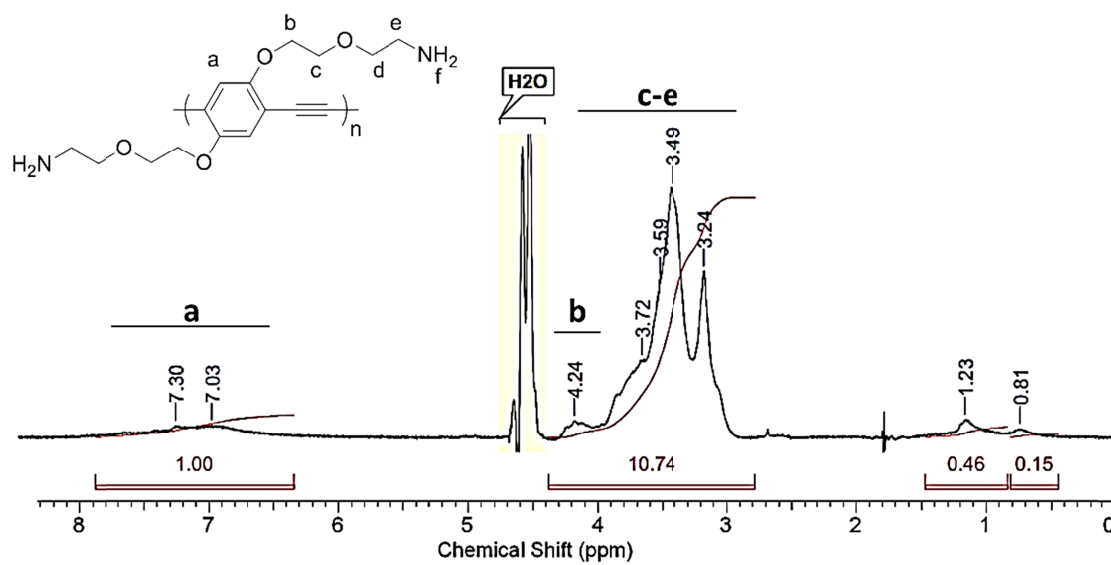


Fig S-3. ¹H-NMR for polymer CPN-2 in D₂O

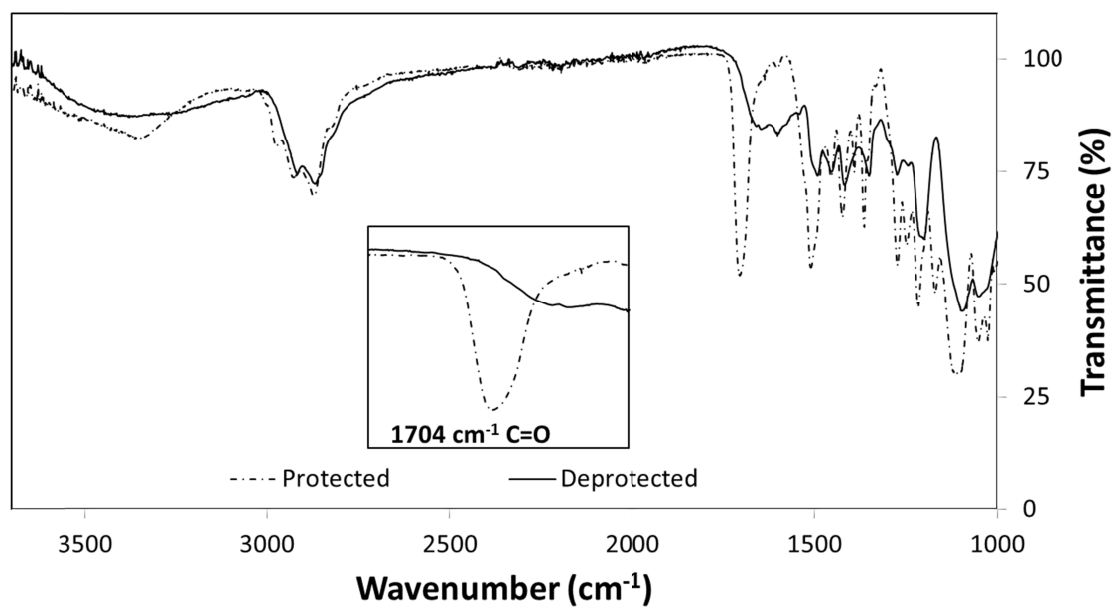


Fig S-4. FT-IR for polymer **P2** (Neat)

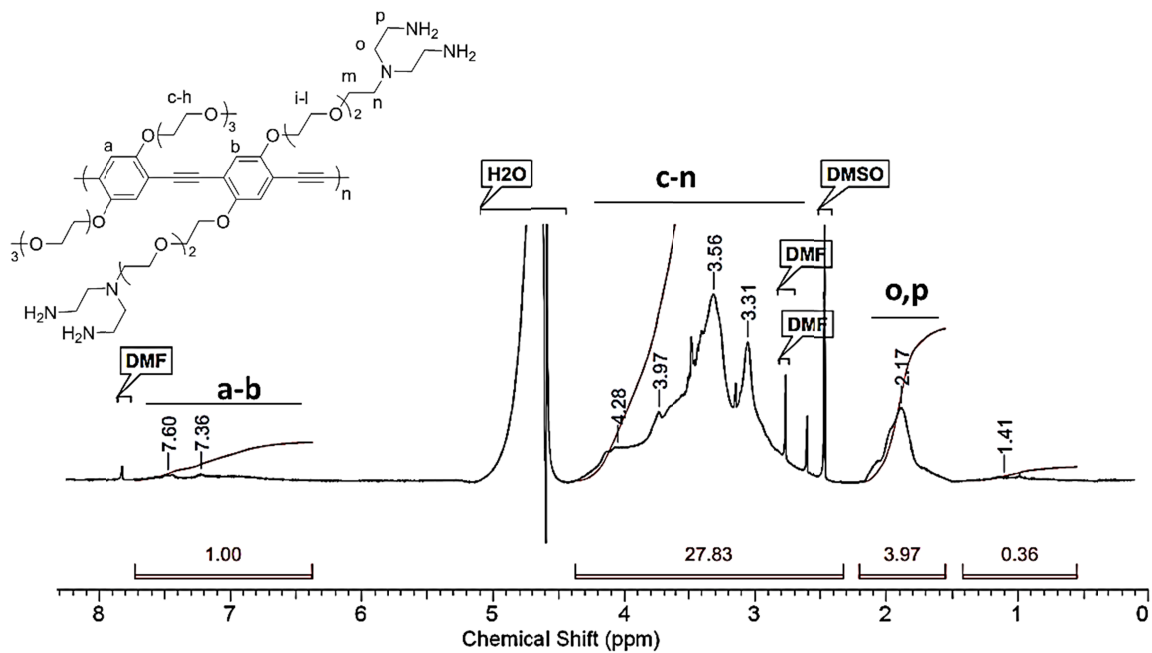


Fig S-5. $^1\text{H-NMR}$ for polymer CPN-3 in D_2O

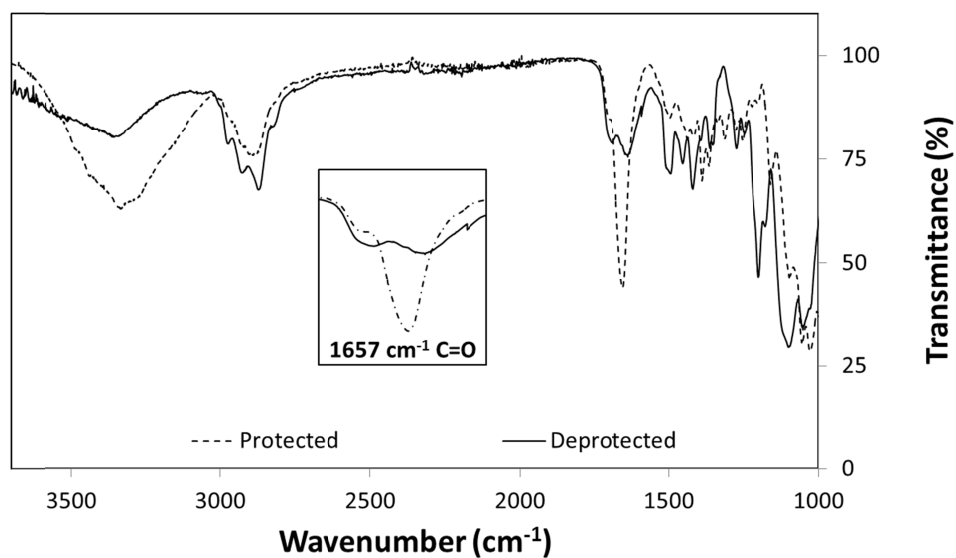


Fig S-6. FT-IR for polymer P3 (Neat)

Gel Permeation Chromatography. Samples dissolved in THF were filtered through a Teflon (PTFE) syringe filter (0.25 μm) three times. The average molecular weight (M_n) and polydispersity index ($\text{PDI} = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC) against polystyrene standards using a Shimadzu high performance liquid chromatography (HPLC) system fitted with PLgel 5 μm MIXED-D columns and SPD-20A ultraviolet– visible (UV–Vis) detector.

Dyniamic Light Scattering (DLS). Hydrodynamic radii and Zeta potentials (ZP) were determined by dynamic light scattering technique using a Zetasizer nano–ZS (Zen 3600, Malvern Instruments Ltd.). Three polymer aliquots of 500 μM CPN were independently prepared by filtering through a Teflon (PTFE) syringe filter (0.45 μm). DLS samples loaded in a low volume (1.5 mL) disposable cuvette, and Zeta potential samples into disposable capillary cell. Averages and standard deviations reported in the main text. Figures S7-14, show representative reports for polymer **CPN-1, -2, -3** and **-4**.

File Name: JM123 & 3-145.dts Dispersant Name: Water
Record Number: 46 Dispersant RI: 1.330
Material RI: 1.59 Viscosity (cP): 0.8872
Material Absorbtion: 0.01 Measurement Date and Time: Monday, December 17, 2012 ...

System

Temperature (°C): 25.0 Duration Used (s): 30
Count Rate (kcps): 287.6 Measurement Position (mm): 4.65
Cell Description: Low volume disposable sizing ... Attenuator: 9

Results

	Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 60.55	Peak 1: 92.46	94.9	55.58
Pdl: 0.293	Peak 2: 13.82	5.1	3.029
Intercept: 0.920	Peak 3: 0.000	0.0	0.000

Result quality : **Good**

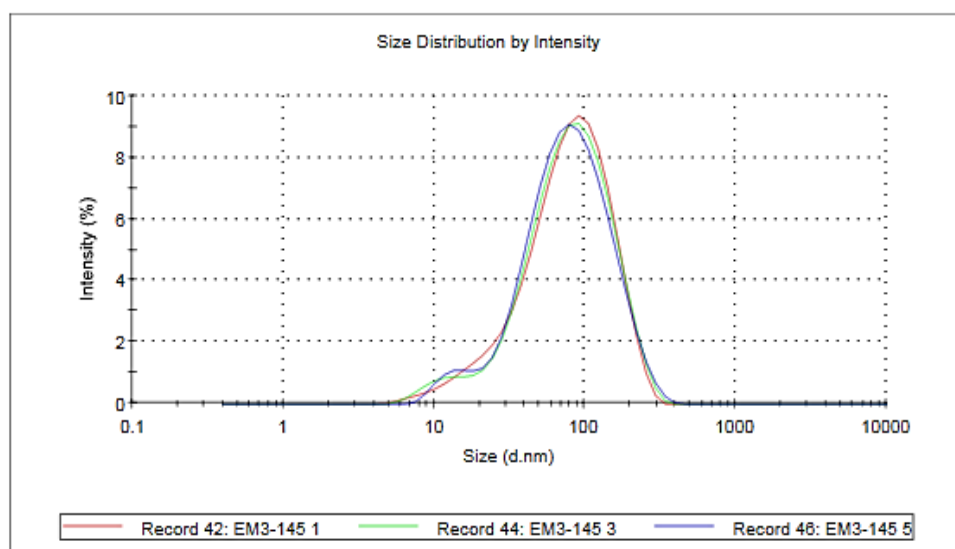


Fig S-7. DLS for polymer CPN-1

File Name: JM123 & 3-145.dts Dispersant Name: Water
Record Number: 13 Dispersant RI: 1.330
Date and Time: Monday, December 17, 2012 5:3... Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 13
Count Rate (kcps): 9.0 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 11

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 20.2	Peak 1: 20.2	99.7	7.73
Zeta Deviation (mV): 7.84	Peak 2: -9.98	0.3	1.78
Conductivity (mS/cm): 0.732	Peak 3: 0.00	0.0	0.00

Result quality : Good

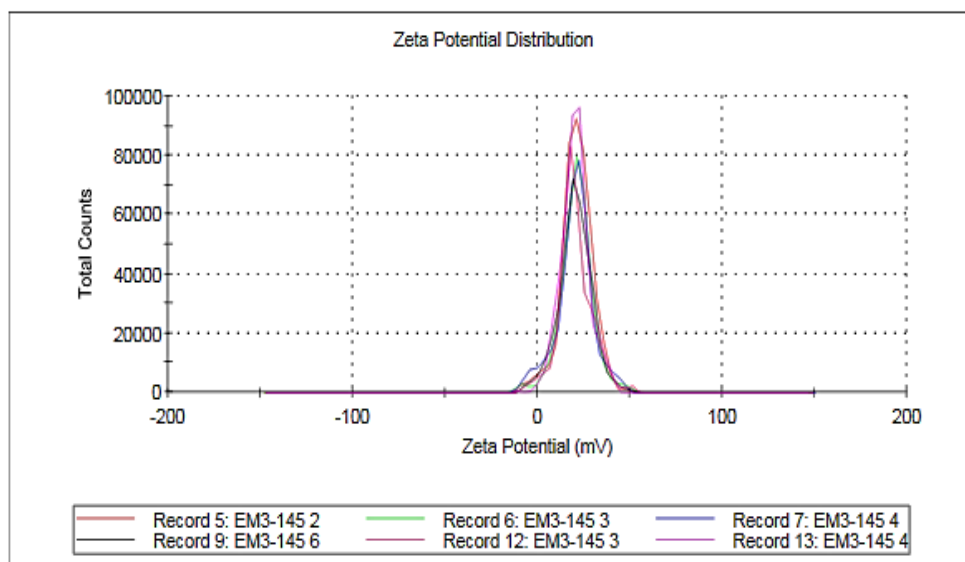


Fig S-8. ZP for polymer CPN-1

File Name: ZR268 Experiment.dts Dispersant Name: Water
Record Number: 17 Dispersant RI: 1.330
Material RI: 1.59 Viscosity (cP): 0.8872
Material Absorbtion: 0.01 Measurement Date and Time: Monday, December 03, 2012 ...

System

Temperature (°C): 25.0 Duration Used (s): 60
Count Rate (kcps): 342.6 Measurement Position (mm): 4.65
Cell Description: Disposable sizing cuvette Attenuator: 8

Results

	Diam. (nm)	% Intensity	Width (nm)
Z-Average (r.nm): 71.22	Peak 1: 92.04	93.5	40.09
Pdl: 0.295	Peak 2: 18.80	6.5	4.213
Intercept: 0.903	Peak 3: 0.000	0.0	0.000

Result quality: **Good**

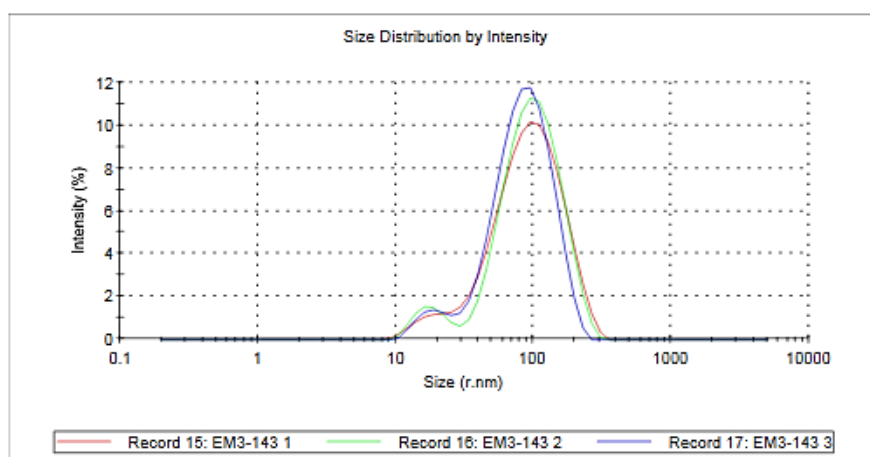


Fig S-9. DLS for polymer CPN-2

File Name: ZR268 Experiment.dts Dispersant Name: Water
Record Number: 30 Dispersant RI: 1.330
Date and Time: Monday, December 03, 2012 4:5... Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 13
Count Rate (kcps): 12.4 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 11

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 42.3	Peak 1: 42.3	100.0	7.70
Zeta Deviation (mV): 7.70	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.0833	Peak 3: 0.00	0.0	0.00

Result quality : Good

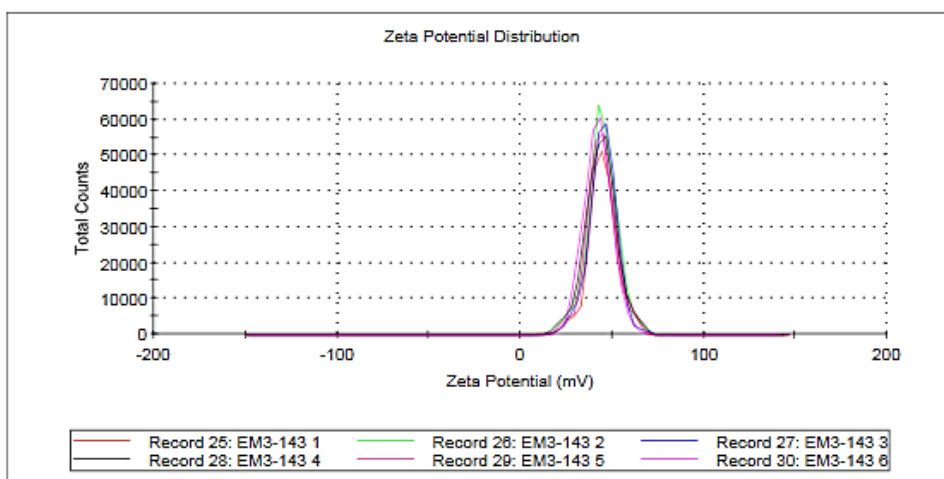


Fig S-10. ZP for polymer CPN-2

File Name: Isotherm studies.dts Dispersant Name: Water
Record Number: 105 Dispersant RI: 1.330
Date and Time: Monday, March 04, 2013 4:50:45 ... Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 32
Count Rate (kcps): 21.7 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 10

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 45.0	Peak 1: 45.0	100.0	14.3
Zeta Deviation (mV): 14.3	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm): 0.263	Peak 3: 0.00	0.0	0.00

Result quality : Good

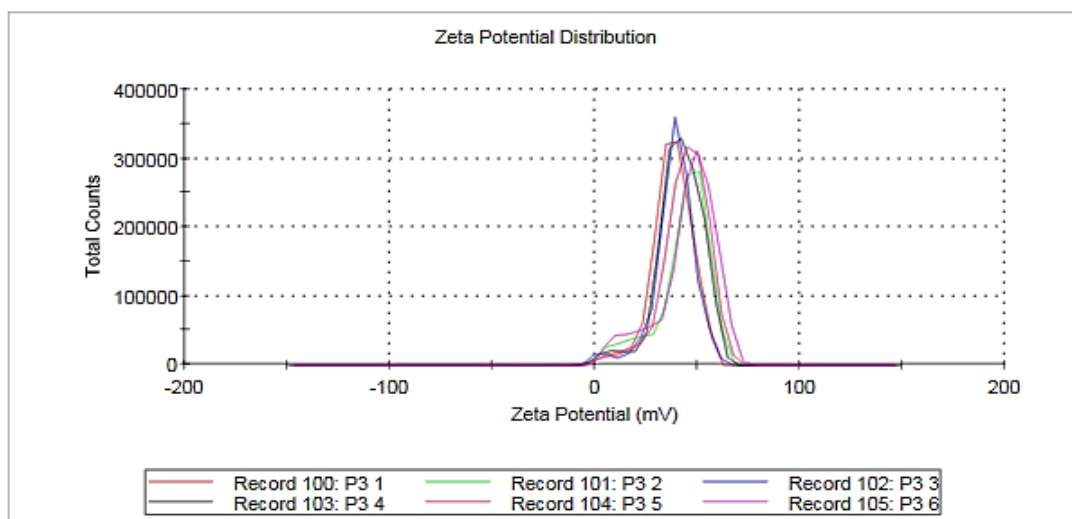


Fig S-11. DLS for polymer CPN-3

File Name: 1mM.dts
Record Number: 419
Material RI: 1.59
Material Absorbance: 0.01
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Monday, January 28, 2013 7:...

System

Temperature (°C): 25.0
Count Rate (kcps): 378.5
Cell Description: Low volume disposable sizing ...
Duration Used (s): 16
Measurement Position (mm): 4.65
Attenuator: 10

Results

	Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm): 84.61	Peak 1: 167.7	85.7	96.47
PdI: 0.538	Peak 2: 22.74	14.3	7.349
Intercept: 0.721	Peak 3: 0.000	0.0	0.000

Result quality: Good

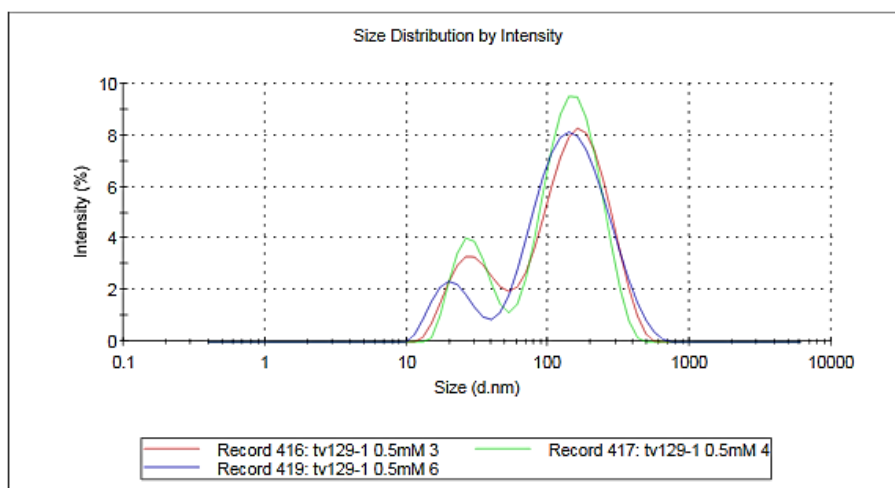


Fig S-13. DLS for polymer **CPN-4**

System

Temperature (°C):	25.0	Zeta Runs:	32
Count Rate (kcps):	17.1	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	11

Results

	Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): 43.8	Peak 1: 45.5	95.2	9.09
Zeta Deviation (mV): 12.0	Peak 2: 7.87	4.8	5.24
Conductivity (mS/cm): 0.134	Peak 3: 0.00	0.0	0.00

Result quality : Good

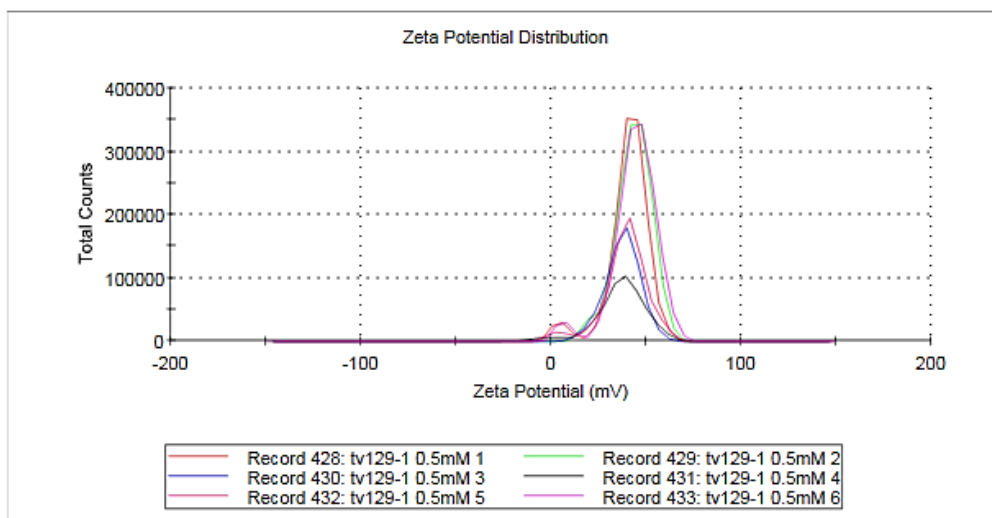


Fig S-14. ZP for polymer CPN-4

Cell Culture.

General. HeLa cells (human cervical cancer, purchased from ATCC), seeded into a 100 x 20mm style sterile tissue culture dish (#353003 BD Falcon, Durham, NC, USA), cultured in minimum essential medium (MEM)/Earle's balanced salt solution (EBSS) (400 μ L, HyClone, SH30024) medium containing 10% fetal bovine serum (FBS) and 100 U/m penicillin for 24 h under 5% CO₂ at 37 °C, and subcultured every 48 hours. pHRhodo Dextran 10kDa (#P10361 Molecular probes, Life technologies, New York, USA) and BODIPY-TR C5 -ceramide-BSA complex (#B34400 Molecular probes, Life technologies, New York, USA) were used for endosome and Golgi apparatus staining, respectively.

Microscopic Imaging. 10,000 HeLa cells were seeded into a glass-bottomed eight-well chamber slide (Lab-Tek Thermo Scientific) and cultured in a minimum essential medium (MEM)/Earle's balanced salt solution (EBSS) (400 μ L) medium containing 10% fetal bovine serum (FBS) and 100 U/m penicillin for 24 h under 5% CO₂ at 37 °C. 80 μ L of 20 μ M CPNs in water was added to the culture medium directly, and the cells were further cultured overnight (final CPN concentration: 4 μ M). For endosome staining, pHRhodo Dextran 10kDa (5 μ M) was incubated for 30 min at 37°C. For Golgi apparatus staining, BODIPY-TR C5 -ceramide-BSA complex (final 10 μ M) was incubated for 30 min at 4 °C. After washing with fresh medium, the cells were further incubated for 30 min at 37 °C. A 1 μ L aliquot of Hoechst (5 μ g/mL) was added to the culture medium and incubated with the cells for 10 min at 37 °C, and washed two times with phosphate buffered saline (PBS). The cells were fixed with 4% paraformaldehyde for 10 min. Fluorescent images of the cells were obtained using a DeltaVision Elite Microscope System (Applied Precision, Issaquah, Washington, USA) equipped with bandpass filters such as blue (410–460 nm, Hoechst), green (500–550 nm, CPNs), and red (595-635nm, Golgi and endosome). Top and bottom of the chosen cells was identified, and a Z-stack plot was imaged for each channel.

Colocalization study. Z-stack microscope images of each sample was obtained as described above. Colocalization analysis was conducted for three independent cells per polymer and per organelle (Golgi apparatus and endosome). Colocalization analysis was conducted using the microscope software (Softworx 5.0 application, Applied Precision, Issaquah, Washington, USA). Region of interest (ROI) was selected to contain all of the cell. Pearsons Correlation Coefficient (PCC) was used to determine colocalization. Negative control of colocalization was performed by analysis of blue and green channels staining the nucleus and CPN, respectively. Three independent images of an entire cell were selected and analysed to increase the analysis objectivity. A representative example is shown in figure S-15.

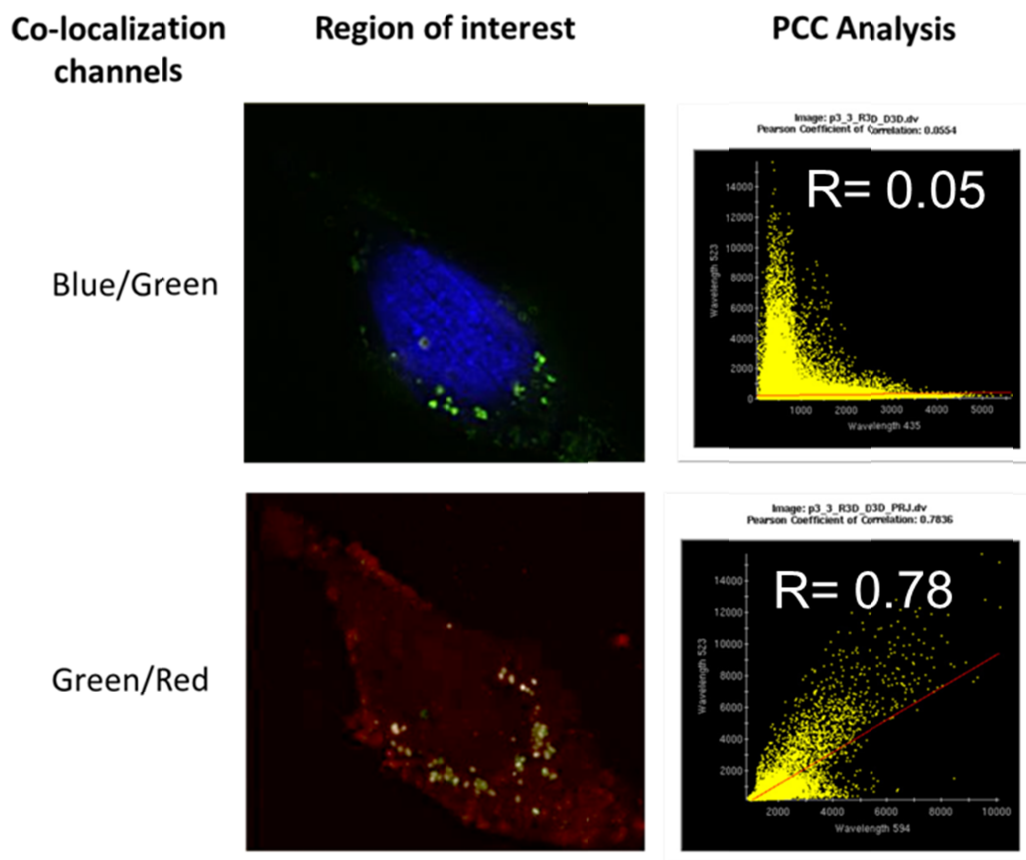


Fig S-15. Example of microscopic images of HeLa cells incubated with **CPN-3** and PCC co-localization analysis of the region of interest. Control and experimental co-localization using blue/green and green/red channels, respectively.

Experimental colocalization PCC values were obtained using the green and red channels staining the CPN and the target organelle (Golgi or endosome). Representative images shown in figures S-16 and S-17. Quantification and statistical analysis was performed by averaging PCC values between the independent cells per polymer and per organelle. One-way ANOVA followed by Tukey means separation method was conducted for PCC colocalization values between samples of same organelle using Origin 8 software.

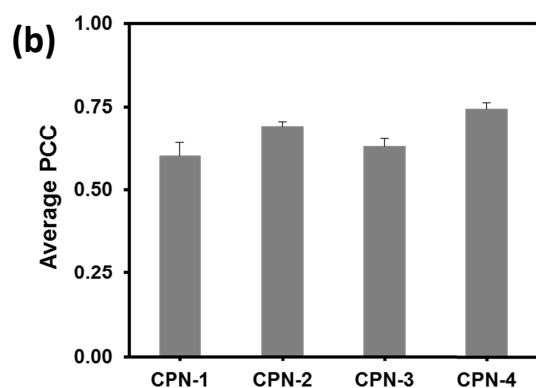
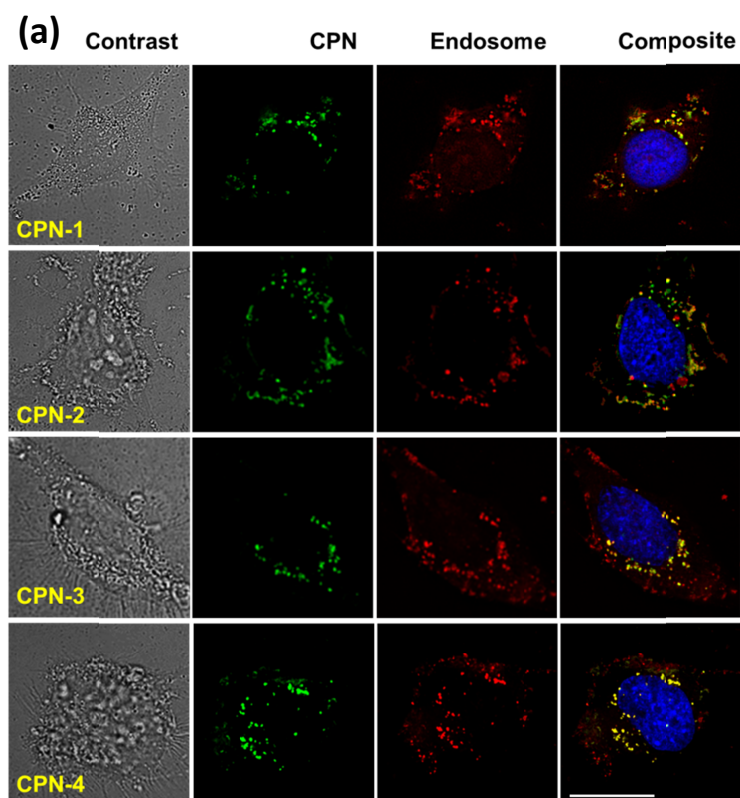


Fig S-16. (a) Microscopic images of HeLa cells incubated with **CPN-1**, **-2**, **-3** and **-4**, respectively, followed by endosome (red) and nucleus (blue) staining. Scale bar is 20 μm . (b) Quantitative analysis of co-localization using PCC algorithm. After overnight incubation, co-localization with endosome is independent on the side chain and backbone structures. Error bar represents \pm standard deviation ($n=3$).

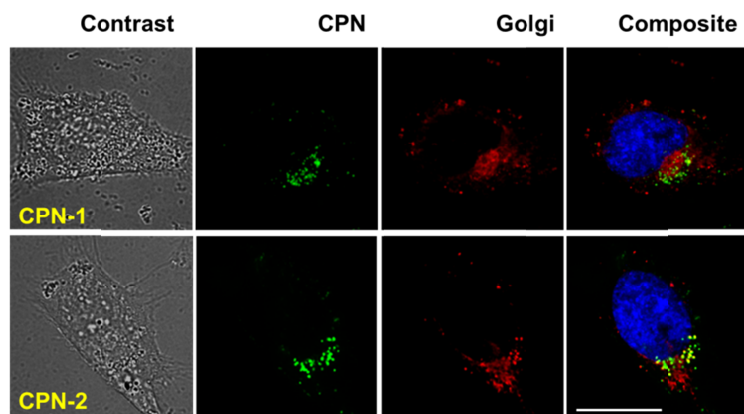


Fig S-17. Microscopic images of HeLa cells incubated with **CPN-1** and **-2**, respectively, followed by Golgi (red) and nucleus (blue) staining. Scale bar is 20 μm . **CPN-2** exhibits higher overlap with Golgi than **CPN-1**.

Toxicity Assay HeLa cells ($\sim 10\,000$ cells/well) in 200 μL of complete medium were seeded into a 96-well plate and cultured for one day in a 5% CO_2 incubator at 37 $^\circ\text{C}$. CPNs with various concentrations (5 to 40 μM) were added and incubated overnight. To measure toxicity, 10 μL of WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] (Cayman Chemical, Ann Arbor, MI, USA) solution was added into each well, and the plate was further incubated for 4 h at 37 $^\circ\text{C}$. Cell viability was compared by measuring absorbance values at 450nm using a microplate well reader (Synergy 2, BioTek, Winooski, VT, USA). Relative cell viability as a function of CPN concentration was obtained by subtracting absorbance values of each sample well with control CPN absorbance at 450 nm.

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