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

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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 Zera 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 (Mn) and polydispersity index (PDI = Mw/Mn) 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. 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 (δ = 4.8 ppm in D₂O). 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 (δ = 2.50 ppm) of the dimethyl sulfoxide (DMSO)-d₆ solvent as an internal standard, or 4.8 ppm for deuterium hydroxide (D₂O) 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.\(^1\) Condensation of 2 and 2,5-diiodohydroquinone in the presence of K$_2$CO$_3$ in 2-butanol resulted in 3. Reaction of 1 with 3 using K$_2$CO$_3$ 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, K$_2$CO$_3$, 2-butanol, reflux, 1 day; iv) K$_2$CO$_3$, MeCN, Reflux, 3 days.

1,4-bis(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-butanol 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 Na$_2$SO$_4$, and evaporated in vaou. The crude product was purified by column chromatography (silica gel, ethyl acetate/ hexane(1:5, v/v) and Yield: 1.5 g (83%).\(^1\)H-NMR(400 MHz) : δ = 7.22 (s, 1H, Ar-H), 4.09 (t, 2H, Ar-O-CH$_2$, J = 6), 3.87 (t, 2H, CH$_2$O, J = 6), 3.82-3.74 (m, 4H, OCH$_2$CH$_2$), 3.69 (t, 2H, CH$_2$Cl), 3.61 (t, 2H, OCH$_2$, J = 6). \(^13\)C-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 C$_{38}$H$_{56}$Cl$_4$O$_6$ [M+Na]$^+$: = 679.9534; found [M+Na]$^+$: 679.9529.
tert-buty1 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 x 3), 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: 0.4 g (44%) Rf = 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), 3.62(br,2H, OCH2), 3.14 (br, 4H, CH2NHBoc, J = 6), 2.68 (br,2H,CH3NH), 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-buty1 2-(2-hydroxy-ethoxy)ethylcarbamate 4, 2-(2-(tert-butoxycarbonylamino)ethoxy)ethyl 4-methylbenzenesulfonate 5, and tert-buty1 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. 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) Boc₂O, MC, room temp., overnight; ii) TsCl, TEA, MC, room temp., overnight; iii) 2,5-diiodohydroquinone, K₂CO₃, DMF, 80°C, 1 day; iv) Trimethylsilylacetylene, Pd(PPh₃)₂Cl₂, Cul, diisopropylamine, THF, 60°C, 12 h; v) K₂CO₃, MeOH, room temp., overnight.

**tert-butyl 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, Pd(PPh₃)₂Cl₂ (0.203 mmol, 146 mg) and Cul (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 methylene chloride, 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%). ¹H-NMR(400 MHz): δ = 7.23 (s, 1H, Ar-H), 5.0 (s, 1H, CH₂-NH), 4.10 (t, 2H, Ar-OCH₂, J = 6), 3.81 (t, 2H, OCH₃, J = 6), 3.83 (t, 2H, OCH₂, J = 6), 3.65 (s, 1H, ArCCH), 3.36 (q, 2H, CH₂NH, J = 6), 1.43 (s, 10H, OC(CH₃)₃), 0.05 (s, 9H, TMS). ¹³C-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 C₃₄H₅₆N₂O₈Si₂ [M+H⁺]: 677.3648; found [M+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 (1.42 mmol, 0.400 g), Potassium carbonate (0.70 mmol, 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^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^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-diodo-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, MC, room temp., overnight; ii) 2,5-diiodohydroquinone, K2CO3, DMF, 80°C, overnight; iii) Trimethylsilylacetylene, Pd(PPh3)4Cl2, CuI, diisopropylamine, THF, 60°C, 12 h; iv) 1M KOH(aq), THF, MeOH, room temp., overnight.](image-url)
Polymer Synthesis.

**General Procedure.** A Schlenk flask was charged with aryl halide monomer (1.0 equiv) and diacetylene 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(Ph3)2Cl2, Cul, 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 (20 mg, 0.044 mmol) in the presence of Pd([Ph3]2Cl2) (3 mg, 0.0044 mmol) and Cul (0.2 mg, 0.0022 mmol) yielded PPE polymer P-1 (32 mg, 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 (400 nm 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 $\lambda_{\text{max}}$ = 433 nm; fluo $\lambda_{\text{max}}$(400 nm ex) = 496 nm; QY = 3% in water. $^1$H-NMR (600 MHz) : $\delta$ = 7.36 (br, 1H, Ar-H), 4.3-3.0 (br, 23H, -OCH$_2$-CH$_2$- chains), 1.30 (s, 0.21H). FT-IR (Neat): v = 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 (41 mg, 0.056 mmol) in the presence of Pd[(PPh$_3$)$_2$Cl$_2$] (4 mg, 0.0056 mmol) and Cul (0.3 mg, 0.0028 mmol) yielded PPE polymer P2 (44 mg, 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 $^1$H-NMR. CPN formation was then carried out as described in general procedure to yield CPN-2.

**CPN-2**: GPC: $M_w$ = 16.9 kDa; $M_n$ = 11.8 kDa; $PDI = 1.43$. UV $\lambda_{\text{max}}$ = 432 nm; fluo $\lambda_{\text{max}}$(400 nm ex) = 475 nm; QY = 37% in DMF. $^1$H-NMR (400 MHz, D$_2$O, $\delta$): 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$_2$O), 3.66 (br, 2H, OCH$_2$), 3.52 (br, 2H, CH$_2$NH), 1.37 (s, 8H, C(CH$_3$)$_3$). FT-IR (Neat): v = 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).

**P3.** Using the general procedure described above, the polymerization of monomer 4 (65 mg, 0.054 mmol) and monomer 13 (32 mg, 0.054 mmol) in the presence of Pd[(PPh$_3$)$_2$Cl$_2$] (8 mg, 0.0054 mmol) and Cul (0.4 mg, 0.0022 mmol) yielded PPE polymer P3 (45 mg, 61%). 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 $^1$H-NMR. CPN formation was then carried out as described in general procedure to yield CPN-3.

**CPN-3**: GPC: $M_w$ = 17.5 kDa; $M_n$ = 10.7 kDa; $PDI = 1.64$. UV $\lambda_{\text{max}}$ = 420 nm; fluo $\lambda_{\text{max}}$(400 nm ex) = 469 nm; QY = 35% in DMF. $^1$H-NMR(400 MHz) : $\delta$ = 7.70-6.95 (br, 1H, Ar-H), 4.0-3.24 (br, 42H, OCH$_2$CH$_3$), 2.30-1.94 (br, 6H, NCH$_2$CH$_2$NH), 1.30 (s, 10H, OC(CH$_3$)$_3$). FT-IR (Neat): v = 3357 (br), 2909 (br), 2916 (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-4**: UV $\lambda_{\text{max}}$ = 420 nm; fluo $\lambda_{\text{max}}$(400 nm ex) = 496 nm; QY = 2% in water. $^1$H-NMR(600 MHz) : $\delta$ = 7.70-6.45 (br, 1H, Ar-H), 4.3-2.5 (br, 28H, OCH$_2$-CH$_2$- chains), 2.17 (br, 4H, NCH$_2$CH$_2$NH) FT-IR...
Polymers. Support Polyme.7 tinging Data.

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

Supporting Data.

Polymer Deprotection. Confirmed using 1H-NMR data by looking at characteristic Boc proton peak (1.36ppm) and ~1700 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. 1H-NMR for polymer CPN-1 in D₂O
**Fig S-2.** FT-IR for polymer P1 (Neat)

**Fig S-3.** $^1$H-NMR for polymer CPN-2 in D$_2$O
Fig S-4. FT-IR for polymer P2 (Neat)
**Fig S-5.** $^1$H-NMR for polymer CPN-3 in D$_2$O

**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 (Mn) 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 μm MIXED-D columns and SPD-20A ultraviolet–visible (UV–Vis) detector.

**Dynamic 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 500μM 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.
Fig S-7. DLS for polymer CPN-1
Fig S-8. ZP for polymer CPN-1
**Fig S-9. DLS for polymer CPN-2**
Fig S-10. ZP for polymer CPN-2
**Fig S-11.** DLS for polymer **CPN-3**
System

Temperature (°C): 25.8  Duration Used (s): 30
Count Rate (kcps): 217.2  Measurement Position (mm): 4.55
Cell Description: Low volume disposable sizing...  Attenuator: 9

Results

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Result quality: Good

Fig S-12. ZP for polymer CPN-3
Fig S-13. DLS for polymer CPN-4
System

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

Results

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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 μL, 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 μL) medium containing 10% fetal bovine serum (FBS) and 100 U/m penicillin for 24 h under 5% CO₂ at 37 °C. 80 μL of 20 μM CPNs in water was added to the culture medium directly, and the cells were further cultured overnight (final CPN concentration: 4 μM). For endosome staining, pHRhodo Dextran 10kDa (5μM) was incubated for 30 min at 37°C. For Golgi apparatus staining, BODIPY-TR C5 -ceramide-BSA complex (final 10 μM) 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 μL aliquot of Hoechst (5 μg/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 ±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 (~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% CO2 incubator at 37 °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 °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.

**References**