Supplementary Information for

Red-Emissive Cell-Penetrating Polymer Dots Exhibiting Thermally Activated Delayed Fluorescence for Cellular Imaging

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Table of Contents

Abbreviations

AFM: atomic force microscopy
CHO: Chinese hamster ovary
DCC: *N,N'*-dicyclohexylcarbodiimide
DIC: differential interference channel
DMAP: dimethylaminopyridine
DMEM: Dulbecco's modified eagle medium
GPC: gel permeation chromatography
Grubbs G3: [1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(benzylidene)bis(3 -bromopyridine)ruthenium(II)
MCS: multi-channel scaling
MEM α: minimum essential medium α
MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NTA: nanoparticle tracking analysis
PBS: phosphate buffered saline
SBR: signal-to-background ratio
SNR: signal-to-noise ratio
TCSPC: time-correlated single photon counting

General Experimental Details

All reactions and manipulations were carried out under a nitrogen atmosphere using standard Schlenk or glovebox techniques unless otherwise stated. Dry solvents were obtained from Sigma-Aldrich, dried using an Innovative Technologies Inc. solvent purification system, and stored under a nitrogen atmosphere unless otherwise stated. Dry CH_2Cl_2 was obtained from Sigma-Aldrich and freshly distilled over P_2O_5 before use. All reagents were obtained from Sigma-Aldrich, TCI, or Oakwood Chemicals and used as received. All ^1H and $^{13}C\{^1H\}$ NMR spectra were obtained on a Bruker AVANCE 400 MHz spectrometer. Absorbance measurements were made on a Cary 60 spectrometer and fluorescence measurements were made on an Edinburgh Instruments FS5 spectrofluorometer. Absolute PL quantum yields were determined using an Edinburgh Instruments SC-30 Integrating Sphere Module. Lifetime measurements were collected using time-correlated single photo counting (TCSPC) or multichannel scaling (MCS) on an Edinburgh Instruments FS5 spectrofluorometer equipped with a TCSPC diode (313 nm) and a Xe microflash lamp. Toluene was used as the solvent for all photophysical experiments unless otherwise stated. Mass spectra were collected on a Katros MS-50 mass spectrometer. Cell images were acquired with an Olympus IX83 inverted epifluorescence microscope. The research-grade microscope was equipped with an X-Cite 120XL metal-halide light source (Excelitas Technologies, Mississauga, ON, Canada), a white-LED transmitted light source, an Orca-Flash 4.0 V2 sCMOS camera (C11440; Hamamatsu Photonics, Hamamatsu, SZK, Japan), motorized filter wheels (Sutter Instruments, Novato, CA), and MetaMorph/MetaFluor software (Molecular Devices, Sunnyvale, CA). CHO, HeLa and HepG2 cells were obtained from UBC Chemistry bioservices.

1,[1] **NAI-THP**,[1] **3**,[2] **4**,[3] and **mCP-OH**[1] were prepared according to literature procedures. Their characterization matches the corresponding literature values; **3** and **4** were used without purification.

Size Exclusion Chromatography (SEC)

SEC experiments were conducted in chromatography-grade THF at concentrations of 0.5 – 2.0 mg mL^{-1} using a Malvern OMNISEC GPC instrument equipped with a Viscotek TGuard column (CLM3008), and Viscotek T3000 (CLM3003) and T6000 (CLM3006) GPC columns packed with porous poly(styrene-*co*-divinylbenzene) particles regulated at 35 °C. Signal response was measured using a differential viscometer, differential refractive index, photodiode array, and light-scattering (90° and 7°) detectors. The interdetector volume was calibrated using a single polystyrene standard with M_n = 101,000 and $Đ$ = 1.04. Molecular

weight for **mCPN** copolymers was determined by triple detection (d*n*/d*c* = 0.259 in THF). Molecular weight for the Boc-protected diblock copolymers was determined by triple detection for the 1st block (d*n*/d*c* = 0.0805 in THF).

Preparation of Polymer Dots

Polymer dot solutions were prepared by dissolving deprotected polymers **BGN_{10}-*b*-M_{20}**, **BGN_{20}-*b*-M_{20}**, **BGN_{10}-*b*-P_{20}**, or **BGN_{10}-*b*-P_{20}** in THF at a concentration of 1 mg mL^{-1}. This solution was injected into 1 mL Milli-Q water under vigorous sonication. The THF was then fully removed under reduced pressure, such that the final concentration of polymer was 1 mg mL^{-1} in water. Polymer dot solutions were stored at 4 °C until use. These solutions were diluted with growth medium for cell studies (MEM-α for CHO and HeLa; DMEM for HepG2).

Nanoparticle Tracking Analysis (NTA) Characterization

The sizes of the Pdots were determined on a Nanoparticle Tracking Analyzer (Malvern NS300) instrument equipped with a 488-nm peak wavelength laser, operating at a maximum power of 45 mW. The Pdots were analyzed using scattering mode (no emission filter).

Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) images were obtained using an Asylum Instruments Cypher S AFM system in tapping mode at scan rates of 0.3 Hz. Samples were prepared by spincoating aqueous solutions of **BGN_{20}-*b*-M_{20}** Pdots onto freshly cleaved highly-oriented pyrolytic graphite (HOPG) at 2500 rpm for 30 s at concentrations of ~0.01 mg mL^{-1}. Samples were placed in a vacuum oven (50 ºC) for at least 15 min before images were obtained using Mikromasch HQ:NSC19/No Al probes, with typical resonance frequencies f = 65 kHz and spring constants k = 0.5 N/m.

Cytotoxicity

Cell viability was measured in triplicate using a standard MTT assay. Cells were seeded in a 96-well plate (1.5×10^4 cells per well) in 100 µL of growth medium and incubated for 24 hours at 37 °C in 5% CO_2 to allow for attachment. Polymer dot solutions, diluted to the desired concentrations with growth medium, were added (100 µL per well) followed by incubation for 24 hours. Then, 50 µL MTT (2.5 mg mL^{-1} in PBS) was added to each well, followed by incubation for 3 hours. The solution was removed from all wells, and 100 µL DMSO was added. The absorbance at 570 nm of each well was measured on a Molecular Devices FilterMax F5 Multi-Mode Microplate Reader. All absorbance values were corrected for baseline absorbance. Cell viability was determined using Equation 1. The same procedure as above was used for testing cell viability after 1 hour, except 20,000 cells were plated per well, to approximate the number of cells that would be present after 48 hours.

$$Cell\ viability\ (\%) = \frac{Mean\ abs.\ incubated\ cells}{Mean\ abs.\ control\ cells} \times 100\% \qquad 1$$

Flow Cytometry

The efficiency of cellular uptake was measured using flow cytometry. Cells were seeded in a 12-well plate (1.0×10^5 cells mL^{-1}; 1 mL per well) and incubated at 37 °C in 5% CO_2 overnight to allow for attachment. Then, polymer dot solutions, diluted to the desired concentration with growth medium, were added (1 mL per well), followed by incubation for 30 minutes. The cells were then washed 3x with 10% medium in PBS, collected with trypsin, and washed 2x further to remove excess trypsin. Cells were stored as a single-cell suspension in 10% medium in PBS at 4 °C until measurement (cells were measured within 2 hours of collecting). The mean fluorescence of cells was measured on a Beckman Coulter CytoFLEX LX flow

cytometer at a rate of 30 μL min^{-1} and compared to the mean fluorescence of control cells. Control cells were subjected to the same washing steps as incubated cells.

Colocalization Experiments

Cells were seeded on gelatin-coated cover slips inside a 12-well plate (50,000 cells mL^{-1}; 1 mL per well) and incubated at 37 °C in 5% CO_2 overnight to allow for attachment. Then, cells were incubated with polymer dot solutions (25 μg mL^{-1}) and Lysotracker Blue DND-22 (50 nM) for 30 minutes at 37 °C in 5% CO_2. All growth medium was subsequently removed, and the cells were washed 3x with PBS. They were then fixed with 4% w/v paraformaldehyde in PBS at room temperature for 15 minutes, followed by 3 washings with PBS to remove excess paraformaldehyde. The coverslips were then transferred onto microscope slides for imaging. Control samples were subjected to the same washing procedures as test samples.

For colocalization experiments, a 350/50 nm bandpass excitation filter, a dichroic mirror with 470 nm transmission cut-on wavelength, a 550 nm longpass emission filter were chosen based on the excitation and emission spectra of the materials. A 405/20 nm bandpass excitation filter, a dichroic mirror with 425nm cut-on wavelength, a 460/50 nm bandpass emission filter were used based on the spectra of Lysotracker blue DMD-22 dye. Images were taken under 100×O objective and processed with ImageJ software. The Pearson's correlation coefficient was calculated using the JACoP tool in ImageJ.[4]

Cellular Imaging

Cells were seeded on gelatin-coated cover slips inside a 12-well plate (50,000 cells mL^{-1}; 1 mL per well) and incubated at 37 °C in 5% CO_2 overnight to allow for attachment. Then, cells were incubated with polymer dot solutions (25 μg mL^{-1}) for 30 minutes at 37 °C in 5% CO_2. All growth medium was subsequently removed, and the cells were washed 3x with PBS. They were then fixed with 4% w/v paraformaldehyde in PBS at room temperature for 15 minutes, followed by 3 washings with PBS to remove excess paraformaldehyde. The coverslips were then transferred onto microscope slides for imaging. Control samples were subjected to the same washing procedures as test samples.

A 350/50 nm bandpass excitation filter, a dichroic mirror with 470 nm transmission cut-on wavelength, a 550 nm longpass emission filter were chosen based on the excitation and emission spectra of the materials. Images were taken under 20× and 60× objective and processed with ImageJ software. Control and test samples were collected with the same microscope settings. SBR and SNR were calculated according to equations 2 and 3, where I = intensity, bg = background, and σ = standard deviation.

$$SBR = \frac{I_{cell} - I_{bg}}{I_{bg}} \quad (2)$$

$$SNR = \frac{I_{cell} - I_{bg}}{\sigma(I_{bg})} \quad (3)$$

Time-gated Measurements

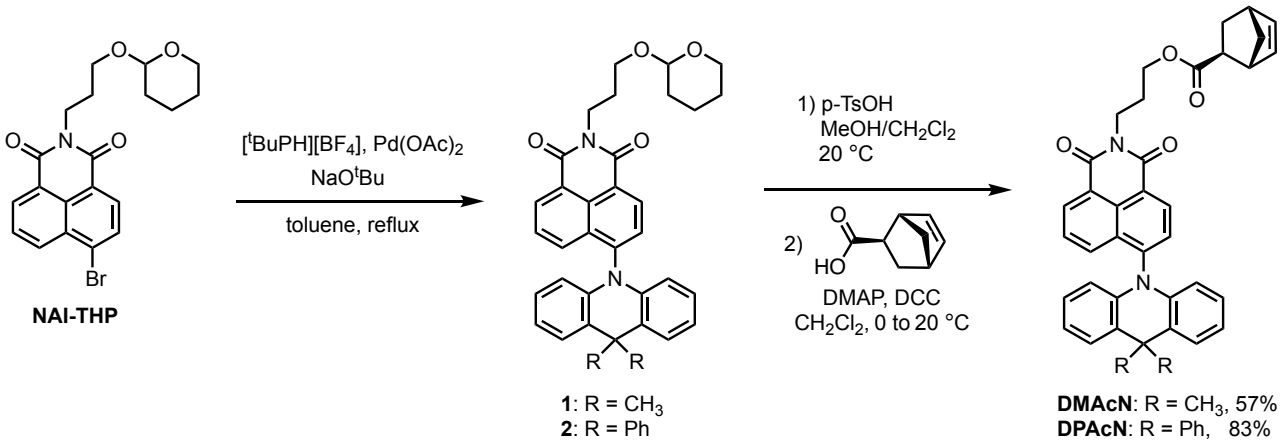
Time-gated measurements were made with an Infinite M1000 Pro plate reader (Tecan). The first set of time-gated emission measurements were made with fluorescein (~1.17 μM in Borate buffer) and Pdots (~1 mg/mL in water) in Borate buffer (50 mM, pH 8.5), either individually or as a mixture (40 μL and 40 μL, respectively). Prompt/time-gated emission measurements had delay times of 0 μs/50 μs after flash excitation and integration times of 500 μs/500 μs. In both cases, the excitation wavelength was 400 ± 10 nm.

The second set of time-gated emission measurements were made with Pdots (40 μL, ~0.33 mg/mL in HEPES buffer (50 mM, pH=7.1)) mixed with bovine serum (40 μL). Prompt/time-gated emission

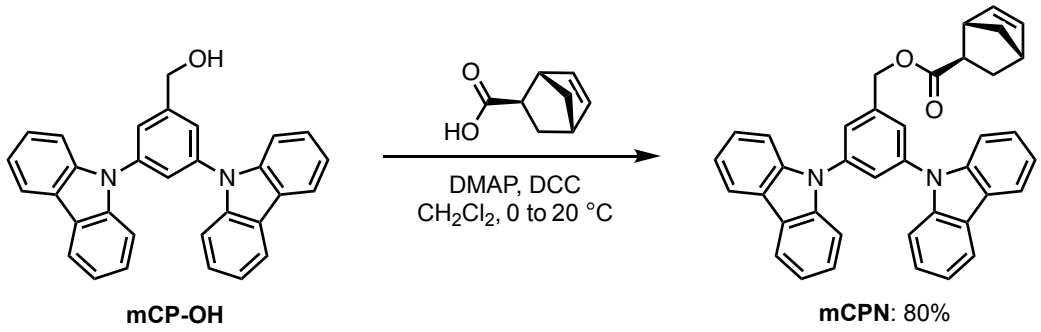
measurements had delay times of 0 μs/50 μs after flash excitation and integration times of 500 μs/500 μs. In both cases, the excitation wavelength was 400 ± 20 nm.

Synthetic Procedures

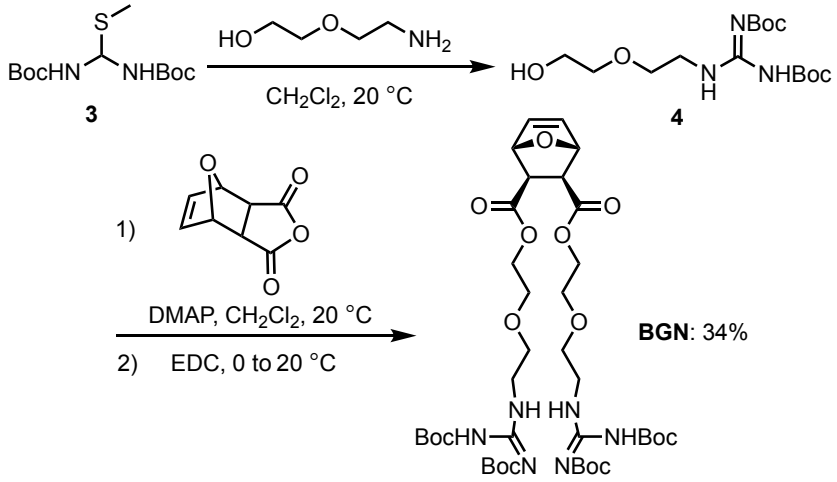
Scheme 1. Synthesis of DMAcN and DPAcN monomers.



Scheme 2. Synthesis of mCPN monomer.



Scheme 3. Synthesis of BGN monomer.



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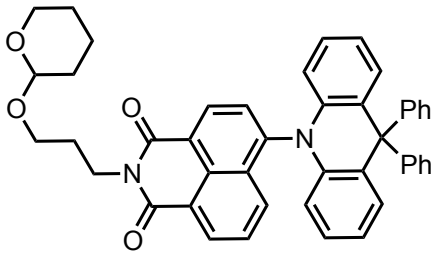
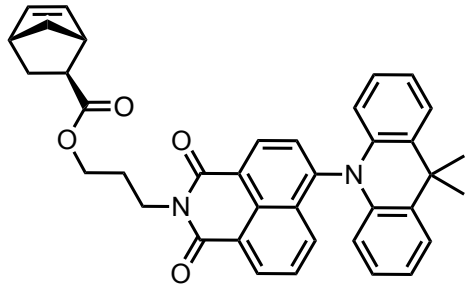


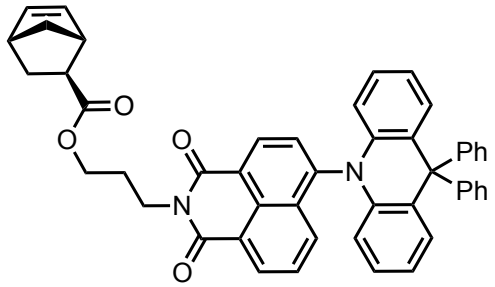
Synthesized from a modified literature procedure.[1] **NAI-THP** (0.778 g, 1.85 mmol, 1 equiv.), 9,9-diphenyl-9,10-dihydroacridine (740 mg, 2.22 mmol, 1.2 equiv.), [^{t}BuPH][BF_4] (64.4 mg, 0.22 mmol, 0.12 equiv.), $Pd(OAc)_2$ (21 mg, 0.09 mmol, 0.05 equiv.) and NaO^{t}Bu (0.44 g, 4.63 mmol, 2.5 equiv.) were combined in a flame-dried 3-neck flask equipped with a stir bar and condenser. The flask with reagents was vacuum/N_2 cycled (4x). Dry, degassed toluene (50 mL) was added and the reaction mixture was heated to reflux overnight. After 16 hours, the reaction mixture was cooled, the toluene removed, and the resulting solid was dissolved in CH_2Cl_2. The organic layer was washed with H_2O (3x) and brine (1x). The organic layer was dried over $MgSO_4$, and the solvent was removed *in vacuo*. Purified on silica using a gradient from 0% to 5% EtOAc in CH_2Cl_2. Product is a yellow crystalline solid (857 mg, 55%). **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 8.79 (d, J = 7.7 Hz, 1H), 8.49 (dd, J = 7.2, 1.2 Hz, 1H), 7.78 (d, J = 7.7 Hz, 1H), 7.40 – 7.28 (m, 6H), 7.23 – 7.12 (m, 3H), 7.08 – 6.88 (m, 9H), 6.35 – 6.24 (m, 2H), 4.61 (t, J = 3.3 Hz, 1H), 4.46 – 4.20 (m, 2H), 3.98 – 3.78 (m, 2H), 3.67 – 3.41 (m, 2H), 2.09 (p, J = 6.8 Hz, 2H), 1.88 – 1.60 (m, 3H), 1.55 – 1.36 (m, 3H). **^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ:** 164.2, 163.8, 146.6, 146.0, 142.9, 141.8, 132.0, 131.6, 131.2, 131.2, 130.6, 130.5, 130.3, 130.2, 129.8, 128.2, 127.9, 127.9, 127.3, 126.9, 126.7, 123.3, 123.0, 121.1, 113.8, 98.8, 65.7, 62.2, 57.1, 38.4, 30.7, 28.4, 25.6, 19.5 ppm. **HRMS (FD/TOF) *m/z* [M]$^{\cdot+}$:** For $[C_{45}H_{38}N_2O_4]^{\cdot+}$ calculated 670.28316; found 670.28467; difference 2.26 ppm.

DMAcN



1 (355 mg, 0.65 mmol, 1 equiv.) and *p*-TsOH•H_2O (24.7 mg, 0.13 mmol, 0.2 eq.) were dissolved in a 1:2 mixture of CH_2Cl_2:MeOH (10 mL), and the reaction was allowed to proceed at room temperature under air. When the reaction was complete by TLC (2 hrs), the solvent was removed *in vacuo*, the crude residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ (aq) (3x). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to afford the crude product, which was used without further purification. To the crude alcohol, *exo*-5-norbornenecarboxylic acid (135 mg, 0.97 mmol, 1.5 equiv.), and DMAP (5 mg, 0.042 mmol, 0.065 equiv.) were combined in a three-neck round-bottom flask equipped with a stir bar and dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was cooled to 0 °C. DCC (161 mg, 0.78 mmol, 1.2 equiv.) was added at 0 °C and the reaction was allowed to warm to room temperature. The reaction was stirred overnight, before being filtered and washed with CH_2Cl_2. The filtrate was evaporated and purified over silica with a gradient of 100% CH_2Cl_2 to 5% EtOAc in CH_2Cl_2. The product was then recrystallized from CH_2Cl_2/hexanes to obtain pure product as an orange-red crystalline solid (215 mg, 57%). **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 8.82 (d, *J* = 7.7 Hz, 1H), 8.64 (dd, *J* = 7.3, 1.2 Hz, 1H), 8.04 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.64 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.54 (dd, *J* = 7.8, 1.5 Hz, 2H), 6.95 (td, *J* = 7.5, 1.3 Hz, 2H), 6.86 (ddd, *J* = 8.7, 7.3, 1.6 Hz, 2H), 6.11 (dd, *J* = 5.7, 3.0 Hz, 1H), 6.02 (dd, *J* = 5.7, 3.1 Hz, 1H), 5.93 (dd, *J* = 8.2, 1.3 Hz, 2H), 4.39 (t, *J* = 7.0 Hz, 2H), 4.32 – 4.20 (m, 2H), 3.04 (dd, *J* = 3.2, 1.6 Hz, 1H), 2.88 (s, 1H), 2.18 (m, 4H), 1.93 (dt, *J* = 11.6, 4.0 Hz, 1H), 1.81 (d, *J* = 33.3 Hz, 6H), 1.51 (s, 1H), 1.38 – 1.29 (m, 2H) ppm. **^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ:** 176.4, 164.1, 163.8, 144.5, 140.2, 138.16, 135.9, 132.8, 132.2, 130.9, 130.4, 130.1, 128.2, 126.9, 126.1, 123.8, 123.0, 121.4, 114.2, 62.7, 46.7, 46.6, 43.3, 41.8, 38.0, 36.2, 32.9, 32.2, 30.5, 27.6 ppm. **HRMS (ESI/TOF) *m/z* [M]$^{\cdot+}$:** For $[C_{38}H_{34}N_2O_4]^{\cdot+}$ calculated 582.2519; found 582.2521; difference 0.40 ppm.

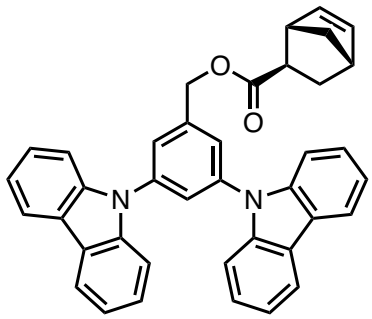
DPAcN



2 (402 mg, 0.60 mmol, 1 equiv.) and *p*-TsOH•H_2O (23 mg, 0.12 mmol, 0.2 eq.) were dissolved in a 1:2 mixture of CH_2Cl_2:MeOH (10 mL), and the reaction was allowed to proceed at room temperature under air. When the reaction was complete by TLC (2 hrs), the solvent was removed *in vacuo*, the crude residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$ (aq) (3x). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to afford the crude product, which was used without further purification. To the crude alcohol, *exo*-5-norbornenecarboxylic acid (124 mg, 0.89 mmol, 1.5 equiv.), and DMAP (5 mg, 0.039 mmol, 0.065 equiv.) were combined in a three-neck round-bottom flask equipped with a stir bar and dissolved in dry CH_2Cl_2 (10 mL). The reaction mixture was cooled to 0 °C. DCC (148 mg, 0.72 mmol, 1.2 equiv.) was added at 0 °C and the reaction was allowed to warm to room temperature. The

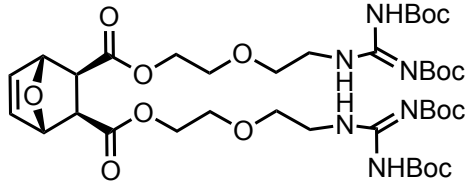
reaction was stirred overnight, before being filtered and washed with CH_2Cl_2. The filtrate was evaporated and purified over silica with 100% CH_2Cl_2. The product was then recrystallized from CH_2Cl_2/hexanes to obtain pure product as an orange crystalline solid (349 mg, 83%). **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 8.79 (d, *J* = 7.7 Hz, 1H), 8.49 (d, *J* = 7.2 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.43 – 6.87 (m, 18H), 6.28 (d, *J* = 8.1 Hz, 2H), 6.10 (dd, *J* = 5.7, 3.0 Hz, 1H), 6.01 (dd, *J* = 5.7, 3.0 Hz, 1H), 4.35 (t, *J* = 7.0 Hz, 2H), 4.25 (t, *J* = 6.2 Hz, 2H), 3.04 (s, 1H), 2.88 (s, 1H), 2.16 (m, 2H), 1.92 (m, 1H), 1.61 (s, 1H), 1.52 (d, *J* = 8.5 Hz, 1H), 1.39 – 1.23 (m, 2H) ppm. **^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ:** 176.3, 164.1, 163.7, 146.6, 146.0, 143.1, 141.8, 138.1, 135.9, 132.1, 131.7, 131.2, 130.7, 130.5, 130.3, 130.2, 129.9, 128.2, 127.9, 127.3, 126.9, 126.7, 123.1, 122.8, 121.1, 113.8, 62.6, 57.1, 46.7, 46.5, 43.3, 41.8, 37.9, 30.4, 27.6 ppm. **HRMS (ESI/TOF) *m/z* [M]$^{\bullet+}$:** For $[C_{48}H_{38}N_2O_4]^{\bullet+}$ calculated 706.2832; found 706.2833; difference 0.24 ppm.

mCPN



mCP-OH (3.34 g, 7.62 mmol, 1.0 equiv.), *exo*-5-norbornenecarboxylic acid (1.59 g, 11.42 mmol, 1.5 equiv.), and DMAP (60.5 mg, 0.50 mmol, 0.065 equiv.) were combined in a three-neck round-bottom flask equipped with a stir bar and dissolved in dry CH_2Cl_2 (100 mL). The reaction mixture was cooled to 0 °C. DCC (1.89 g, 9.14 mmol, 1.2 equiv.) was added at 0 °C and the reaction was allowed to warm to room temperature. The reaction was stirred overnight, before being filtered and washed with CH_2Cl_2. The filtrate was evaporated and purified over silica with 1:1 hexanes/CH_2Cl_2. The product was then recrystallized from CH_2Cl_2/hexanes to obtain pure product as a white fluffy solid (3.39 g, 80%). **^{1}H NMR (400 MHz, $CDCl_3$)** δ: 8.16 (d, *J* = 7.7 Hz, 4H), 7.79 (t, *J* = 2.0 Hz, 1H), 7.70 (d, *J* = 1.9 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 4H), 7.50 – 7.40 (m, 4H), 7.37 – 7.28 (m, 4H), 6.21 – 6.06 (m, 2H), 5.37 (s, 2H), 3.12 (d, *J* = 3.0 Hz, 1H), 2.95 (s, 1H), 2.36 (ddd, *J* = 9.0, 4.5, 1.5 Hz, 1H), 2.00 (dt, *J* = 11.9, 4.0 Hz, 1H), 1.60 – 1.56 (m, 1H), 1.48 – 1.37 (m, 2H) ppm. **^{13}C NMR (101 MHz, $CDCl_3$)** δ: 176.1, 140.6, 140.4, 139.7, 138.3, 135.8, 126.3, 124.8, 124.6, 123.8, 120.6, 120.6, 109.8, 65.3, 46.8, 46.6, 43.3, 41.8, 30.7 ppm. **HRMS (ESI/TOF) *m/z* [M]$^{\bullet+}$**: For $[C_{39}H_{30}N_2O_2]^{\bullet+}$ calculated 558.2307; found 558.2306; difference -0.29 ppm.

Bisguanidinylnorbornene (**BGN**)



4 (6.4 g, 18.4 mmol, 1.9 equiv.), exo-3a,4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3-dione (1.61 g, 9.7 mmol, 1.0 equiv.), and DMAP (118 mg, 0.97 mmol, 0.1 equiv.) were combined in dry CH_2Cl_2 (30 mL) in a Schlenk flask equipped with a stir bar. The reaction was stirred at room temperature overnight. The reaction was then cooled to 0 °C, and EDC•HCl (1.86 g, 9.7 mmol, 1.0 equiv.) was added in 1 portion, after which the reaction was stirred at room temperature overnight. The reaction was diluted with CH_2Cl_2, washed with $KHSO_4$ (10% aq.) and sat. $NaHCO_3$ (aq.), dried over $MgSO_4$, and concentrated *in vacuo*. The product was purified with an alumina plug (CH_2Cl_2) and silica (gradient of 9:1 hexanes/EtOAc to 1:1 hexanes/EtOAc) to give a white solid (2.80 g, 34%). **^{1}H NMR (400 MHz, $CDCl_3$) δ:** 11.48 (s, 2H), 8.59

(s, 2H), 6.44 (s, 2H), 5.28 (s, 2H), 4.43 – 4.12 (m, 4H), 3.65 (dt, J = 20.4, 4.3 Hz, 12H), 2.86 (s, 2H), 1.49 (d, J = 4.6 Hz, 36H) ppm. **$^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ:** 171.3, 163.4, 156.1, 152.8, 136.5, 82.8, 80.5, 79.1, 69.1, 68.5, 64.1, 46.7, 40.4, 28.2, 28.0 ppm. **HRMS (FD/TOF) *m/z* [M + H]$^+$:** For $[C_{38}H_{63}N_6O_{15}]^+$ calculated 843.43514; found 843.43487; difference -0.32 ppm.

Preparation of **mCPN$_{90}$-*co*-DMAcN$_{10}$**

mCPN (207.9 mg, 0.37 mmol, 90 equiv.) and **DMAcN** (24.9 mg, 0.04 mmol, 10 equiv.) were added to a 4 mL vial (equipped with a teflon stir bar and cap), dissolved in CH_2Cl_2 (1.3 mL), and cooled to -20 °C under N_2 atmosphere. Grubbs G3 (300 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.004 mmol, 1 equiv.) was then added rapidly in one portion. Once the polymer had reached 99% conversion by ^{1}H NMR (approximately 24 h) ethyl vinyl ether (100 μL) was added and the reaction was stirred for 15 min. The reaction was warmed to room temperature (~20 °C) and the crude polymer was precipitated into 50 mL of methanol. The precipitated product was isolated by vacuum filtration. The crude solid was purified by preparatory scale SEC using THF, concentrated *in vacuo*, and reprecipitated in methanol from minimal CH_2Cl_2. The pure polymer was isolated by vacuum filtration to obtain an orange powder (104.4 mg, 45%). **DMAcN** doping $\%_{(NMR)}$ = 10 mol%; $M_{n\,(target)}$ = 56,900; $M_{n\,(GPC)}$ = 54,900; $Đ_{(SEC)}$ = 1.08.

Preparation of **mCPN$_{91}$-*co*-DPAcN$_9$**

mCPN (209.7 mg, 0.37 mmol, 91 eq.) and **DPAcN** (25.2 mg, 0.04 mmol, 9 eq.) were added to a 4 mL vial (equipped with a teflon stir bar and cap), dissolved in CH_2Cl_2 (1.3 mL), and cooled to -20 °C under N_2 atmosphere. Grubbs G3 (300 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.004 mmol, 1 equiv.) was then added rapidly in one portion. Once the polymer had reached 99% conversion by ^{1}H NMR (approximately 24 h) ethyl vinyl ether (100 μL) was added and the reaction was stirred for 15 min. The reaction was warmed to room temperature (~20 °C) and the crude polymer was precipitated into 50 mL of methanol. The precipitated product was isolated by vacuum filtration. The crude solid was purified by preparatory scale SEC using THF, concentrated *in vacuo*, and reprecipitated in methanol from minimal CH_2Cl_2. The pure polymer was isolated by vacuum filtration to obtain an orange powder (98.6 mg, 42%). **DPAcN** doping $\%_{(NMR)}$ = 9 mol%; $M_{n\,(target)}$ = 57,300; $M_{n\,(GPC)}$ = 63,700; $Đ_{(SEC)}$ = 1.08.

General Procedure A: Preparation **BGN$_{10}$-*b*-M$_{20}$**

BGN (50.0 mg, 0.06 mmol, 10 eq.) was added to a 4 mL vial (equipped with a teflon stir bar and cap), dissolved in CH_2Cl_2 (85 μL). Grubbs G3 (215 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.006 mmol, 1 equiv.) was then added rapidly in one portion. After 3 hours, the reaction was cooled to -20 ºC. Separately, **mCPN** (59.3 mg, 0.11 mmol, 17.9 equiv.) and **DMAcN** (7.4 mg, 0.013 mmol, 2.1 equiv.) were brought into the glovebox and dissolved in CH_2Cl_2 (571 μL). This solution was cooled to -20 ºC, and added in one portion to the polymerization vial. Once this block had reached 99% conversion by ^{1}H NMR (approximately 24 h), ethyl vinyl ether (100 μL) was added and the reaction was stirred for 15 min. The reaction was warmed to room temperature (~20 ºC) and the crude polymer was precipitated into 50 mL of methanol. The precipitated product was isolated by vacuum filtration. The crude solid was purified by preparatory-scale SEC using THF, concentrated *in vacuo*, and then stirred overnight in 1:1 MeOH/0.2 M HCl (aq.) to remove the protecting groups. The polymer was then purified by dialysis (Spectra/Por molecularporous membrane tubing, MWCO=3.5 kD) in Milli-Q water to remove any small molecules, then collected by lyophilization. The polymer molecular weights and dispersities were characterized before deprotection, as the Boc-protected polymers had better solubility in organic solvents. The molecular weights for the 1st block were calculated from SEC, and the 2nd block were calculated by ^{1}H NMR by using the relative integrations of the peaks at 7.98 and 8.56 ppm. Yield: 65.3 mg, 56%, orange powder. $M_{n\,(target)}$ = 11,300; $M_{n\,(SEC)}$ = 10,700; $Đ_{(SEC)}$ = 1.17.

Preparation of **BGN$_{20}$-*b*-M$_{20}$**

Prepared according to General Procedure A, using the following amounts: **BGN** (50.0 mg, 0.06 mmol, 20 equiv.), Grubbs G3 (108 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.003 mmol, 1 equiv.), **mCPN** (29.7 mg, 0.05 mmol, 17.9 equiv.), **DMAcN** (3.71 mg, 0.006 mmol, 2.1 equiv.). Yield: 40.3 mg, 48%, orange powder. $M_{n\,(target)}$ = 11,300; $M_{n\,(SEC)}$ = 12,300; $Đ_{(SEC)}$ = 1.26.

Preparation of **BGN$_{10}$-*b*-P$_{20}$**

Prepared according to General Procedure A, using the following amounts: **BGN** (50.0 mg, 0.06 mmol, 10 equiv.), Grubbs G3 (215 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.006 mmol, 1 equiv.), **mCPN** (59.3 mg, 0.11 mmol, 17.9 equiv.), **DPAcN** (7.4 mg, 0.01 mmol, 1.8 equiv.). Yield: 64.0 mg, 55%, yellow powder. $M_{n\,(target)}$ = 11,300; $M_{n\,(SEC)}$ = 11,400; $Đ_{(SEC)}$ = 1.25.

Preparation of **BGN$_{20}$-*b*-P$_{20}$**

Prepared according to General Procedure A, using the following amounts: **BGN** (50.0 mg, 0.06 mmol, 20 equiv.), Grubbs G3 (108 μL, 1 mg mL^{-1} in CH_2Cl_2, 0.003 mmol, 1 equiv.), **mCPN** (29.7 mg, 0.053 mmol, 17.9 equiv.), **DPAcN** (3.71 mg, 0.005 mmol, 1.8 equiv.). Yield: 38.2 mg, 46%, yellow powder. $M_{n\,(target)}$ = 11,300; $M_{n\,(SEC)}$ = 11,400; $Đ_{(SEC)}$ = 1.26.

Supplementary Tables

Table S1. Mean cellular fluorescence in B610-ECD-A channel, from flow cytometry.

	HeLa			CHO			HepG2		
Control	5930			3900			11050		
Pdot Conc. (μg/mL)	5	10	25	5	10	25	5	10	25
BGN$_{10}$-*b*-M$_{20}$	9280	13200	15400	5440	6630	8330	28500	40640	69500
BGN$_{20}$-*b*-M$_{20}$	8370	9280	10130	9200	10400	12400	14480	18150	22170
BGN$_{10}$-*b*-P$_{20}$	8880	9800	11340	8600	10200	11800	29200	43980	66300
BGN$_{20}$-*b*-P$_{20}$	8290	8640	9000	9570	10470	15750	16310	19630	24100

Supplementary Figures

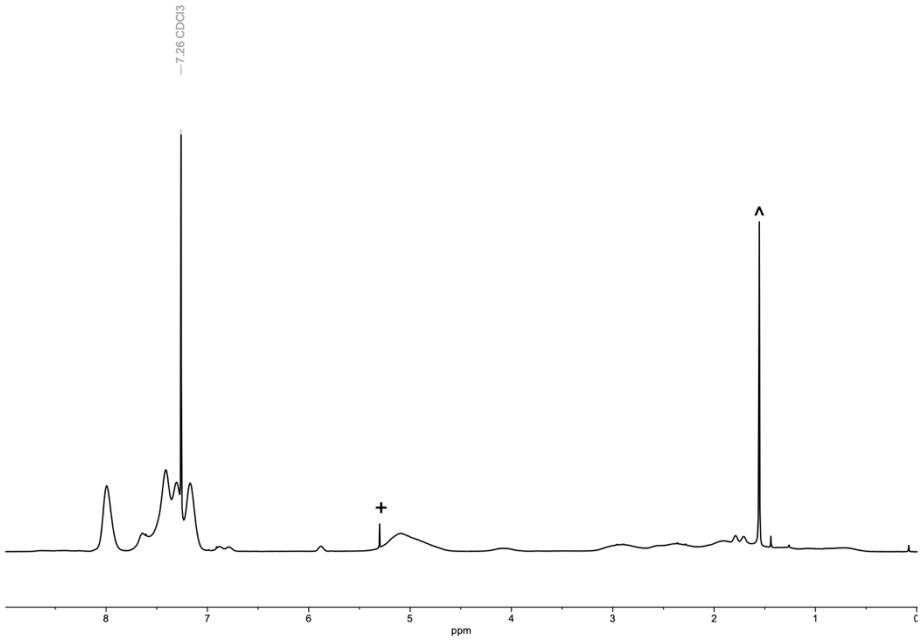


Figure S1. ^{1}H NMR (400 MHz) spectrum of **mCPN$_{90}$-*co*-DMAcN$_{10}$** in $CDCl_3$. ^ = H_2O, + = CH_2Cl_2.

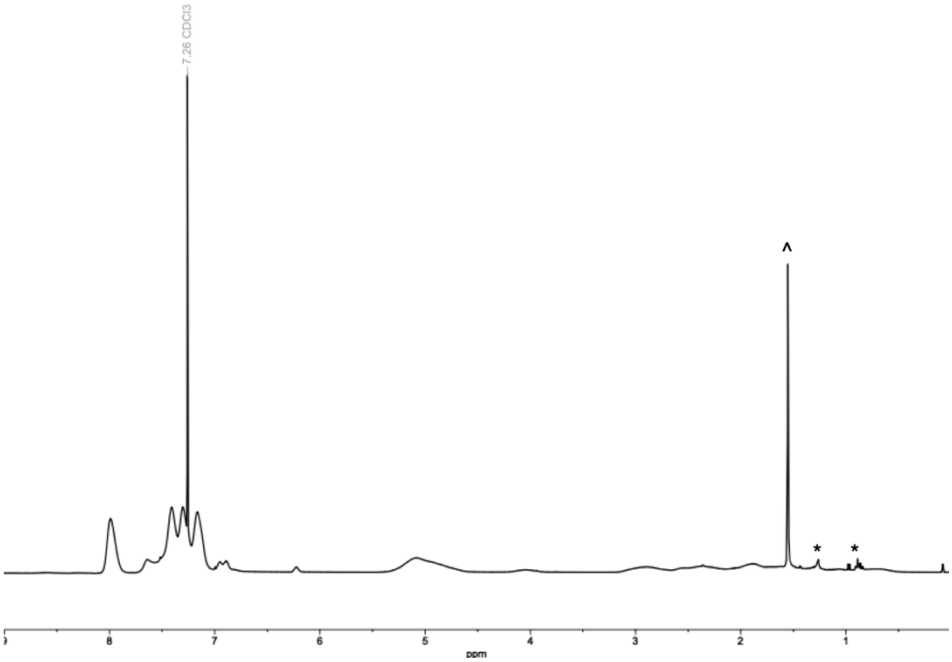


Figure S2. ^{1}H NMR (400 MHz) spectrum of **mCPN$_{91}$-*co*-DPAcN$_{9}$** in $CDCl_3$. ^ = H_2O, * = *n*-hexanes

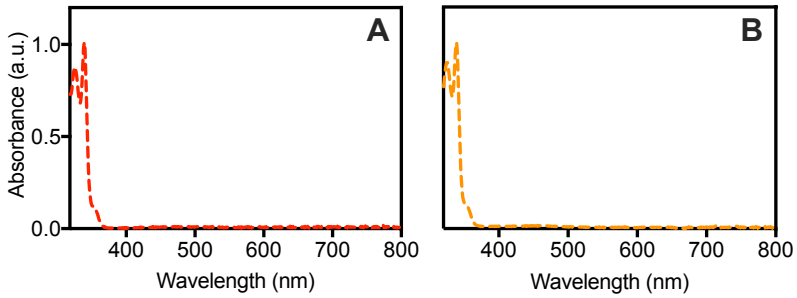


Figure S3. Absorbance spectra of (A) **mCPN$_{90}$-*co*-DMAcN$_{10}$** and (B) **mCPN$_{91}$-*co*-DPAcN$_{9}$** in toluene at 0.01 mg mL^{-1}.

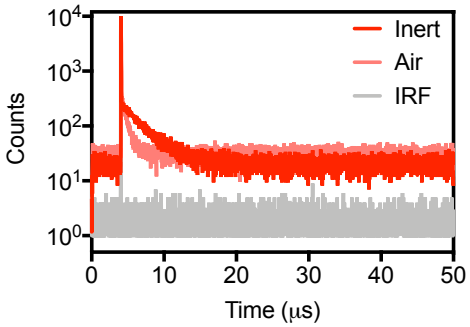


Figure S4. Solution-state photoluminescence lifetimes of **mCPN$_{90}$-*co*-DMAcN$_{10}$** in toluene at 0.01 mg mL^{-1}. λ_{ex} = 313 nm.

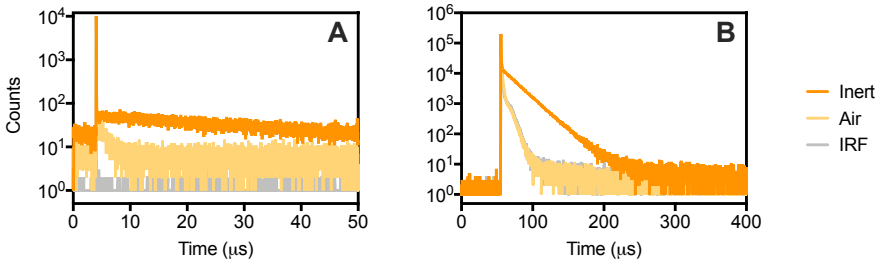


Figure S5. Solution photoluminescence lifetimes of **mCPN$_{91}$-*co*-DPAcN$_{9}$** using (A) TCSPC or (B) MCS in toluene at 0.01 mg mL^{-1}. λ_{ex} = 313 nm.

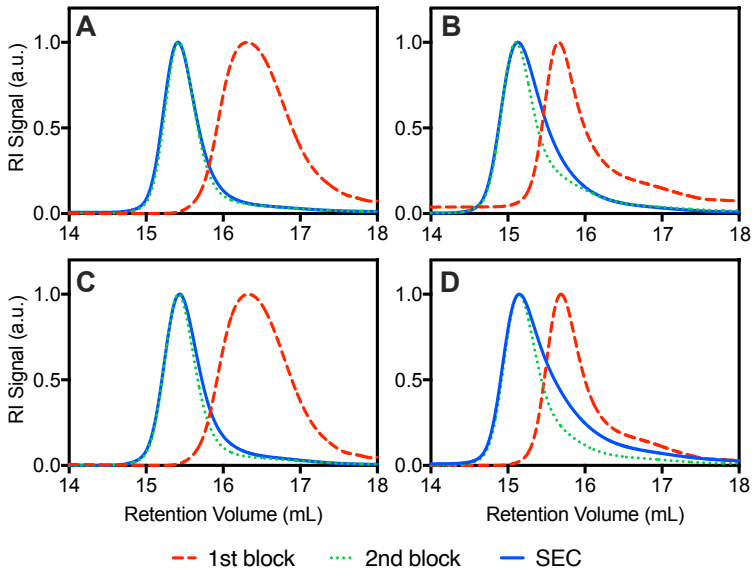


Figure S6. SEC traces of Boc-protected diblock copolymers showing aliquot after 1st block, aliquot after 2nd block, and the purified polymer. (A) **BGN$_{10}$-*b*-M$_{20}$**, (B) **BGN$_{20}$-*b*-M$_{20}$**, (C) **BGN$_{10}$-*b*-P$_{20}$**, and (D) **BGN$_{20}$-*b*-P$_{20}$**.

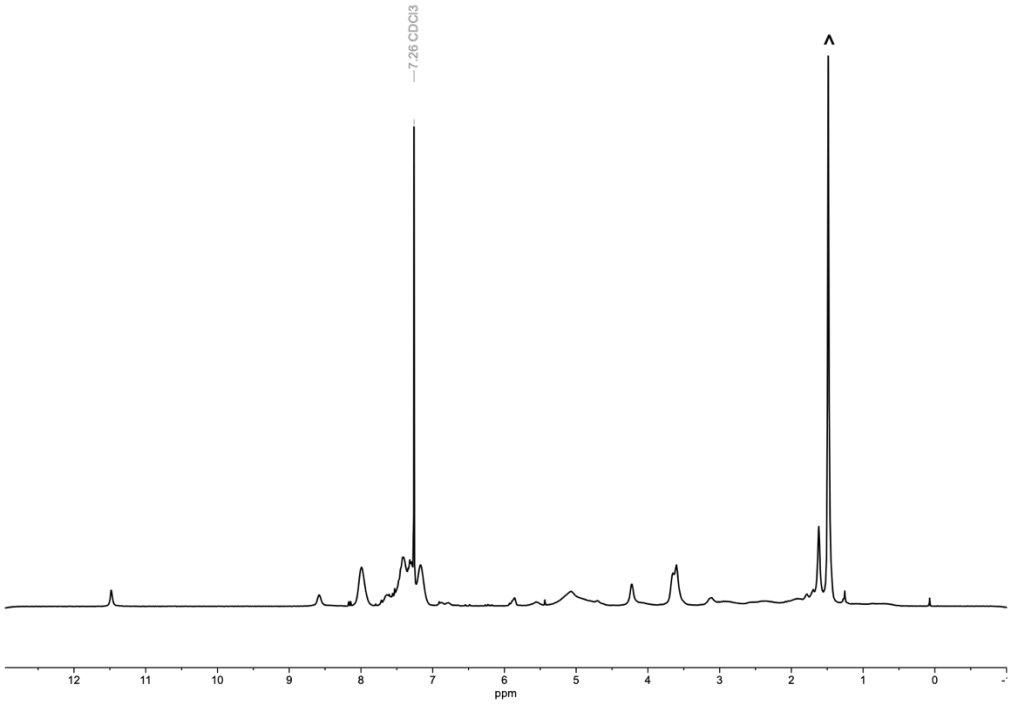


Figure S7. ^{1}H NMR (400 MHz) spectrum of Boc-protected **BGN$_{10}$-*b*-M$_{20}$** in $CDCl_3$. ^ = H_2O

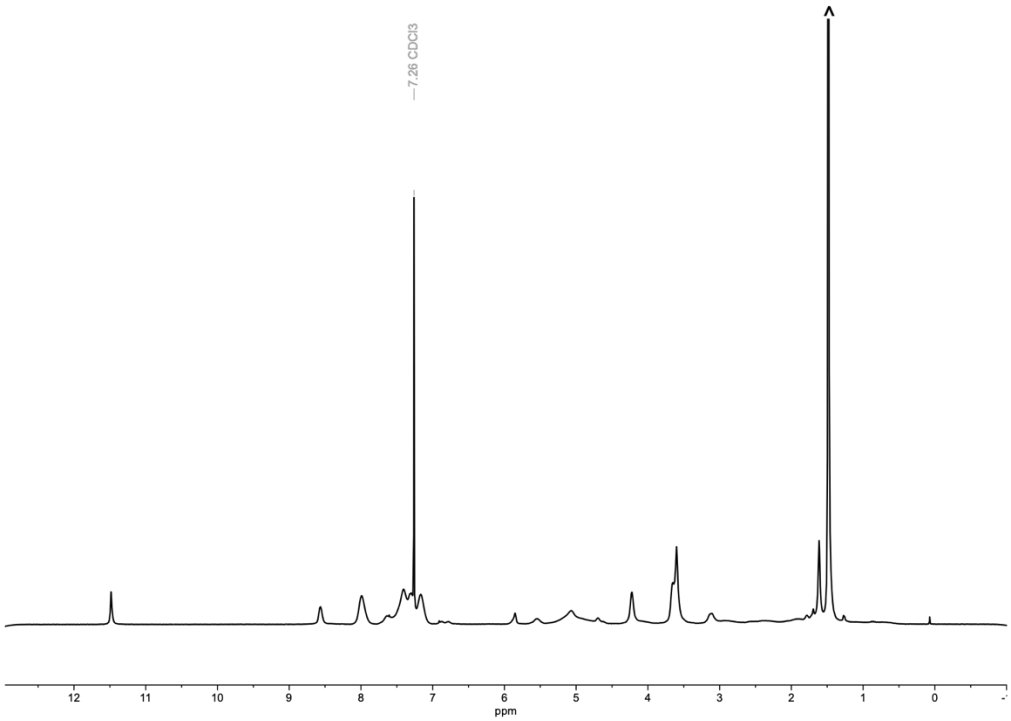


Figure S8. ^{1}H NMR (400 MHz) spectrum of Boc-protected **BGN$_{20}$-*b*-M$_{20}$** in $CDCl_3$. ^ = H_2O

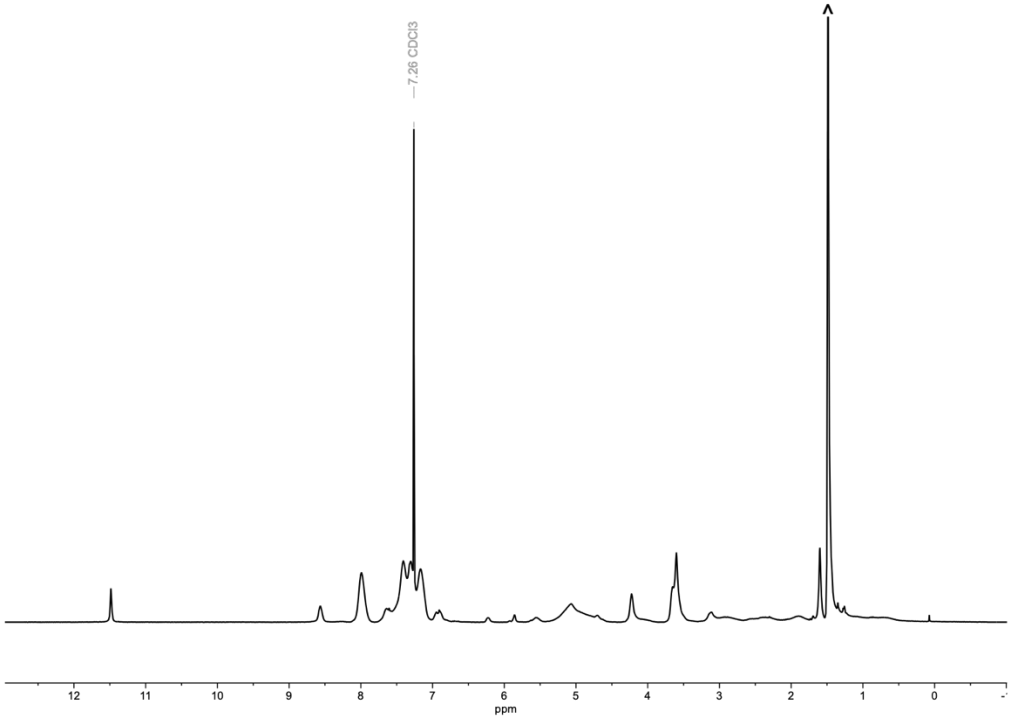


Figure S9. ^{1}H NMR (400 MHz) spectrum of Boc-protected **BGN$_{10}$-*b*-P$_{20}$** in $CDCl_3$. ^ = H_2O

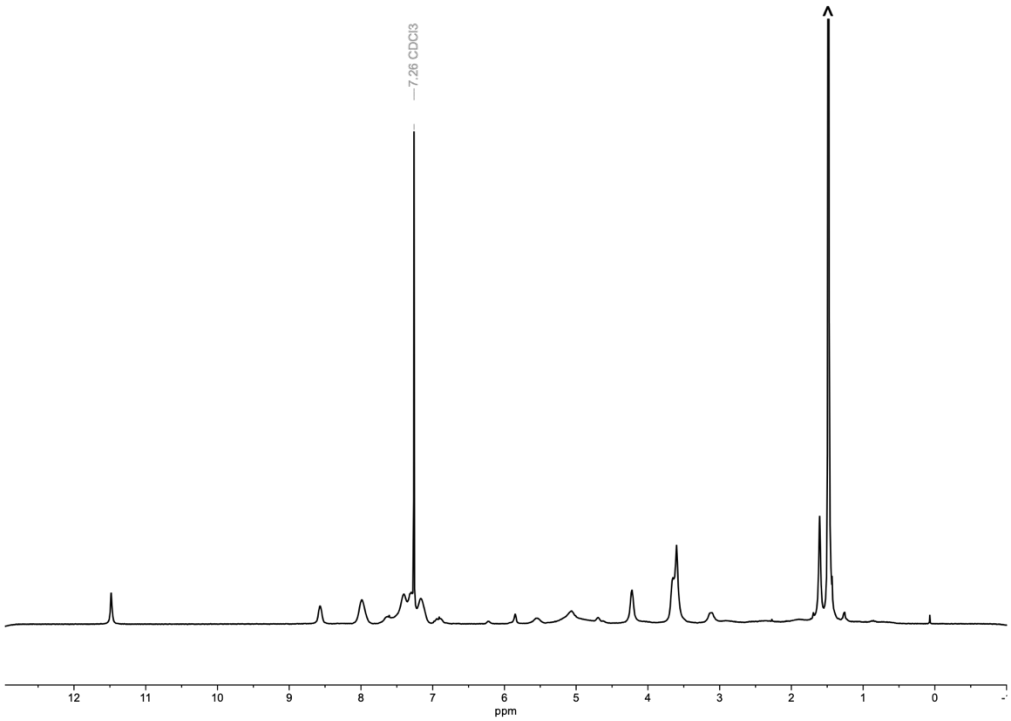


Figure S10. ^{1}H NMR (400 MHz) spectrum of Boc-protected **BGN$_{20}$-*b*-P$_{20}$** in $CDCl_3$. ^ = H_2O

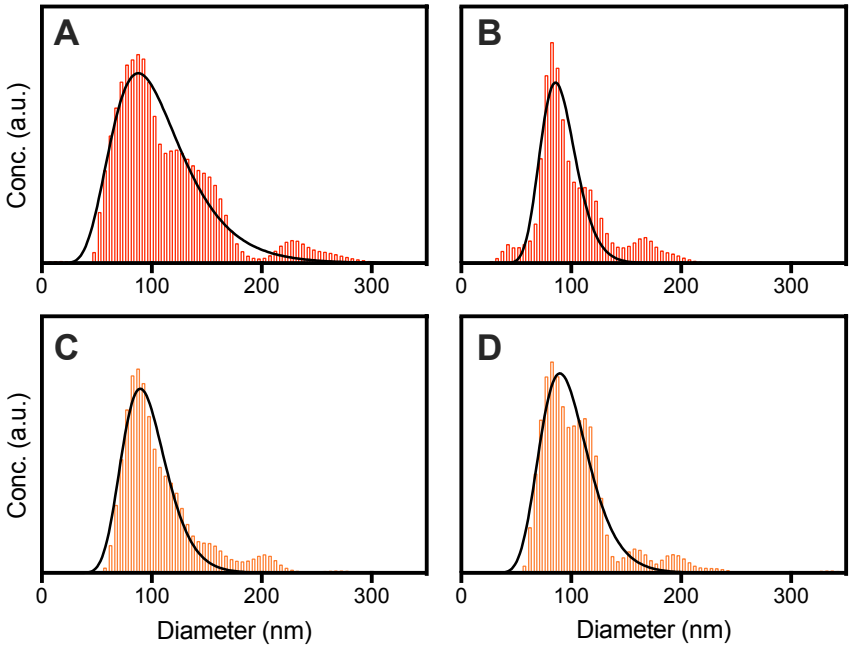


Figure S11. Size distribution of Pdots in aqueous solution at 1 mg mL^{-1} (bar) and curve fit (solid line). (A) **BGN$_{10}$-*b*-M$_{20}$**, (B) **BGN$_{20}$-*b*-M$_{20}$**, (C) **BGN$_{10}$-*b*-P$_{20}$**, and (D) **BGN$_{20}$-*b*-P$_{20}$**.

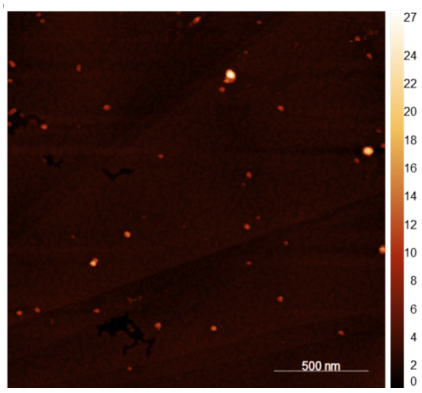


Figure S12. AFM height images of **BGN_{20}-*b*-M_{20}** Pdots on HOPG substrates. Scale bar = 500 nm.

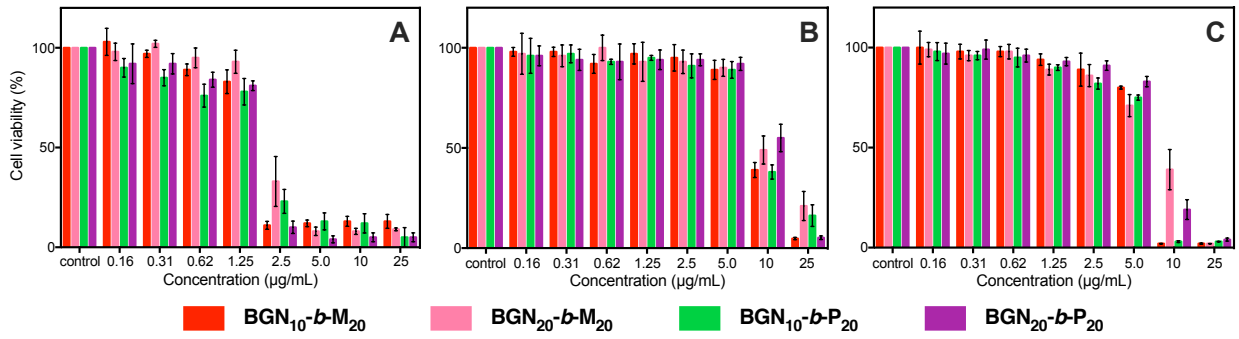


Figure S13. Cell viability of (A) CHO, (B) HeLa, and (C) HepG2 cells after incubation with Pdots for 24 h, measured by MTT assay.

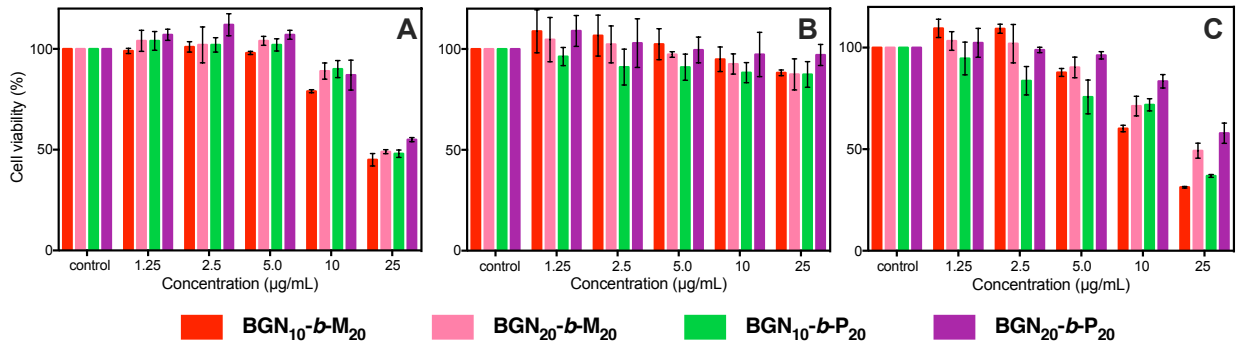


Figure S14. Cell viability of (A) CHO, (B) HeLa, and (C) HepG2 cells after 1 h incubation with Pdot solutions at varying concentrations (0 – 25 μg mL^{-1}); measured by an MTT assay.

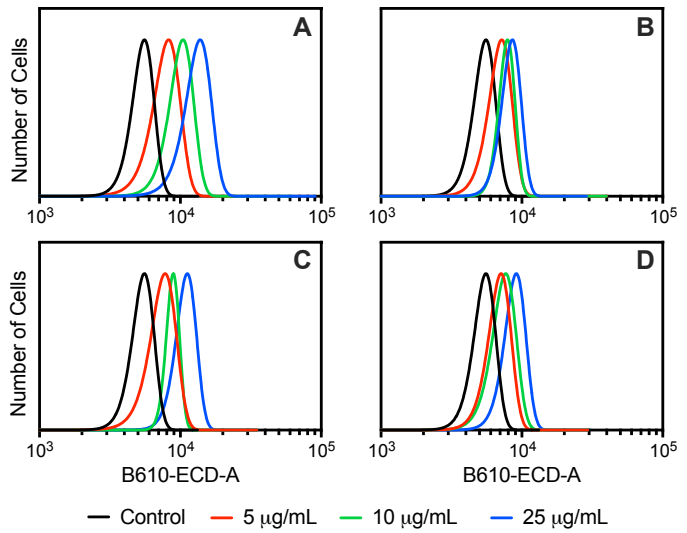


Figure S15. Mean fluorescence of HeLa cells after incubation for 30 min with (A) **BGN$_{10}$-*b*-M$_{20}$**, (B) **BGN$_{20}$-*b*-M$_{20}$**, (C) **BGN$_{10}$-*b*-P$_{20}$**, and (D) **BGN$_{20}$-*b*-P$_{20}$** Pdots at varying concentrations. Measured by flow cytometry.

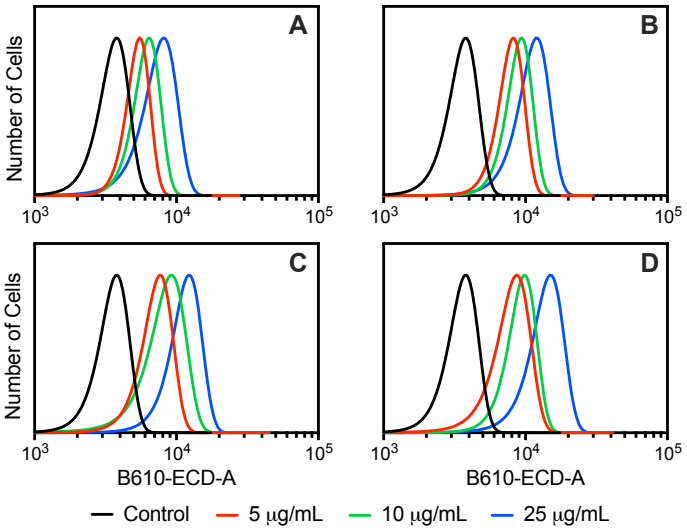


Figure S16. Mean fluorescence of CHO cells after incubation for 30 min with (A) **BGN$_{10}$-*b*-M$_{20}$**, (B) **BGN$_{20}$-*b*-M$_{20}$**, (C) **BGN$_{10}$-*b*-P$_{20}$**, and (D) **BGN$_{20}$-*b*-P$_{20}$** Pdots at varying concentrations. Measured by flow cytometry.

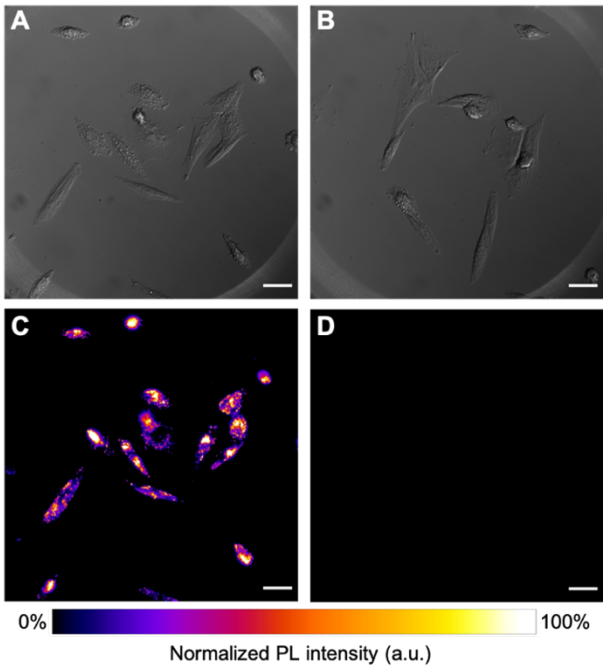


Figure S17. (A, B) Differential interference contrast (DIC) and (C, D) photoluminescence (PL) images of CHO cells (A, C) incubated with **BGN$_{10}$-*b*-M$_{20}$** Pdots and (B, D) control without any incubation with Pdots. PL images were taken with the same microscope settings and adjusted to the same brightness and contrast. Scale bar = 20 μm. For image C: SBR = 2.6 ± 0.2; SNR = 24 ± 2 (from 12 cells).

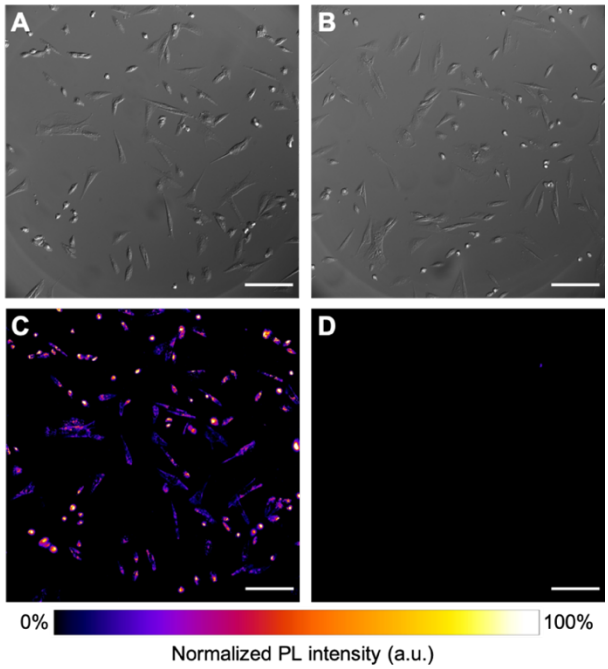


Figure S18. (A, B) DIC and (C, D) photoluminescence images of CHO cells incubated with (A, C) **BGN$_{10}$-*b*-M$_{20}$** Pdots and (C, D) control without any incubation with Pdots. PL images were taken with the same microscope settings and adjusted to the same brightness and contrast. Scale bar = 100 μm. For image C: SBR = 3.2 ± 0.7; SNR = 28 ± 6 (from 74 cells).

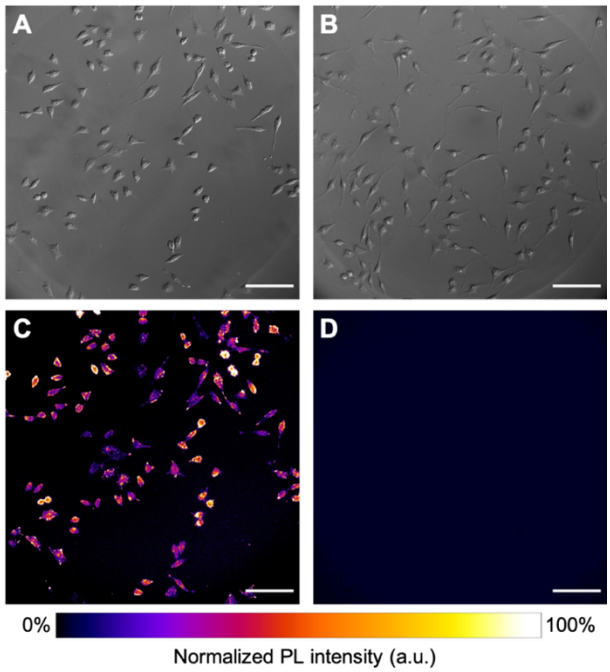


Figure S19. (A, B) DIC and (C, D) photoluminescence images of HeLa cells incubated with (A, C) **BGN$_{10}$-*b*-M$_{20}$** Pdots and (C, D) control without any incubation with Pdots. PL images were taken with the same microscope settings and adjusted to the same brightness and contrast. Scale bar = 100 μm. For image C: SBR = 1.4 ± 0.1; SNR = 16 ± 1 (from 63 cells).

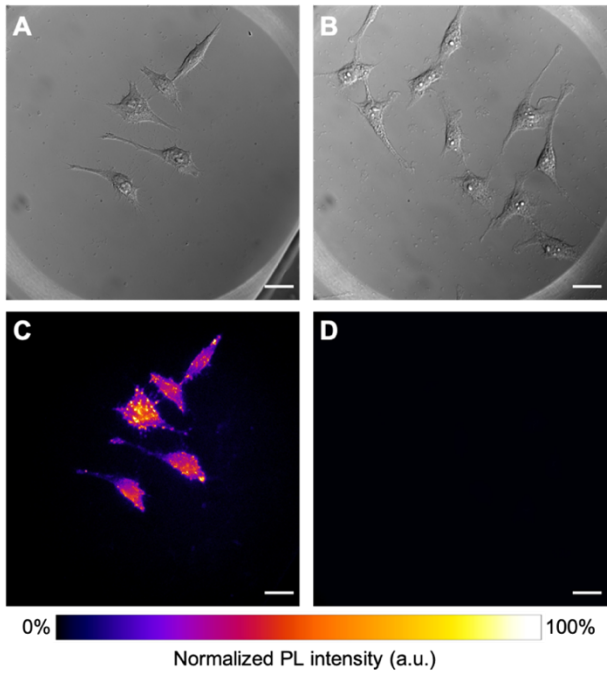


Figure S20. (A, B) DIC and (C, D) photoluminescence images of HeLa cells incubated with (A, C) **BGN$_{10}$-*b*-M$_{20}$** Pdots and (B, D) control without any incubation with Pdots. PL images were taken with the same microscope settings and adjusted to the same brightness and contrast. Scale bar = 20 μm. For image C: SBR = 1.3 ± 0.1; SNR = 14 ± 1 (from 5 cells).

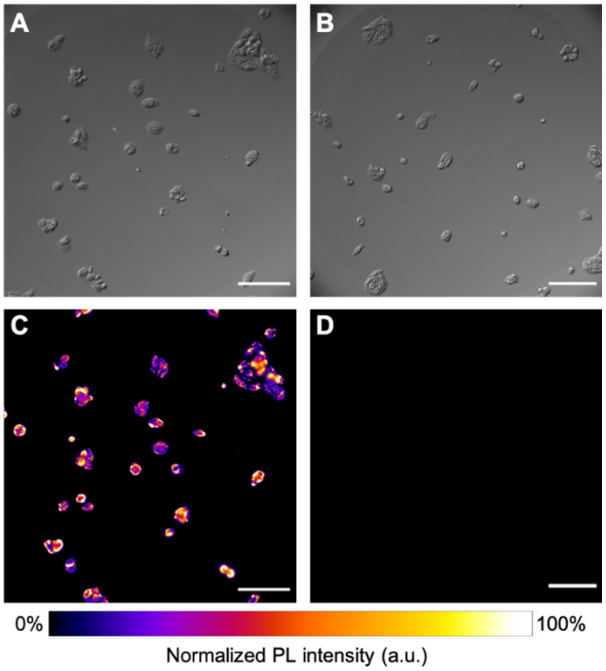


Figure S21. (A, B) DIC and (C, D) photoluminescence images of HepG2 cells incubated with (A, C) **BGN_{10}-*b*-M_{20}** Pdots and (C, D) control without any incubation with Pdots. PL images were taken with the same microscope settings and adjusted to the same brightness and contrast. Scale bar = 100 μm. For image C: SBR = 2.2 ± 0.4; SNR = 28± 5 (from 28 cells).

References

(1) Christopherson, C. J.; Mayder, D. M.; Poisson, J.; Paisley, N. R.; Tonge, C. M.; Hudson, Z. M. 1,8-Naphthalimide-Based Polymers Exhibiting Deep-Red Thermally Activated Delayed Fluorescence and Their Application in Ratiometric Temperature Sensing. *ACS Appl. Mater. Interfaces* **2020**, *12* (17), 20000–20011.

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