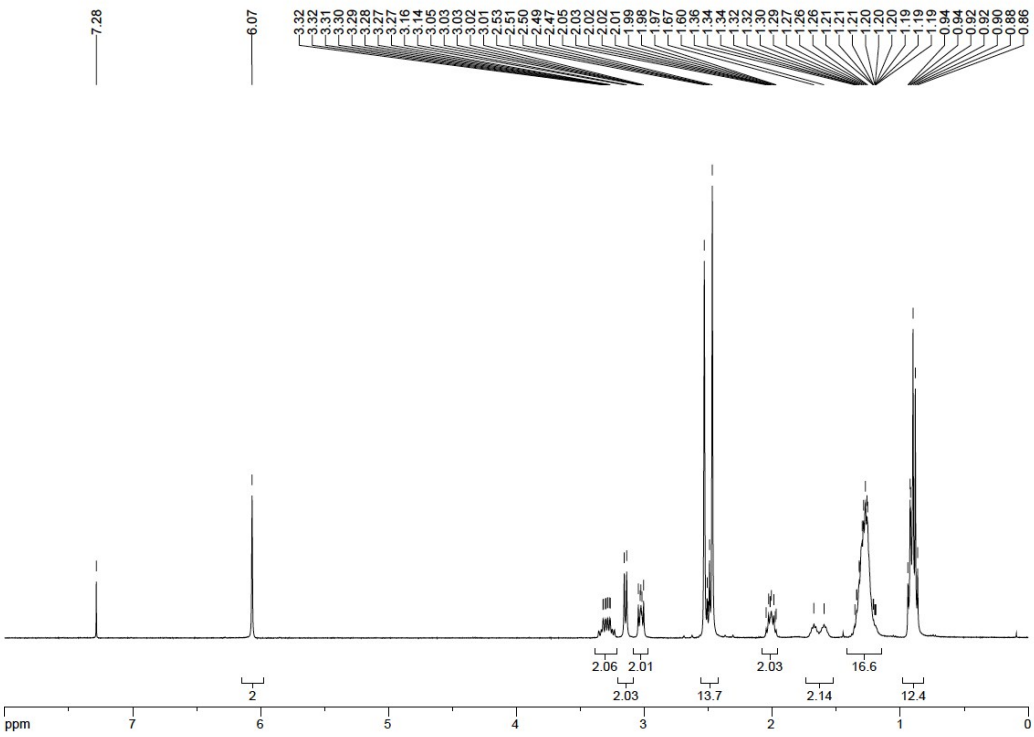
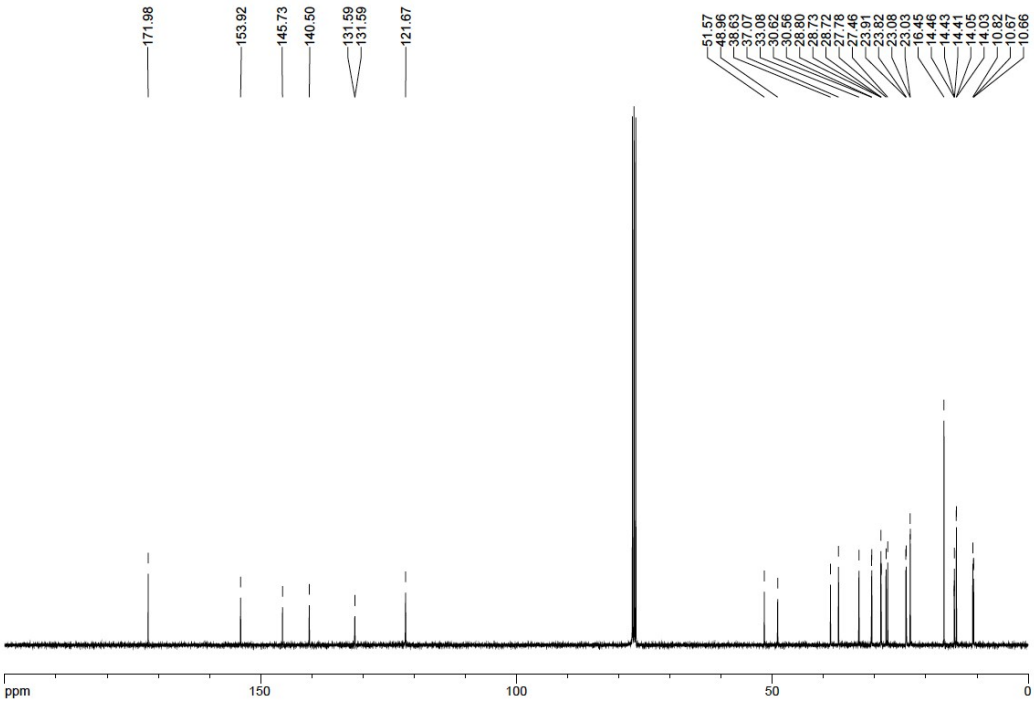
Electronic Supplementary Material (ESI) for Soft Matter.

Lipid-Core/Polymer-Shell Hybrid Nanoparticles: Synthesis and Characterization by Fluorescence Labeling and Electrophoresis

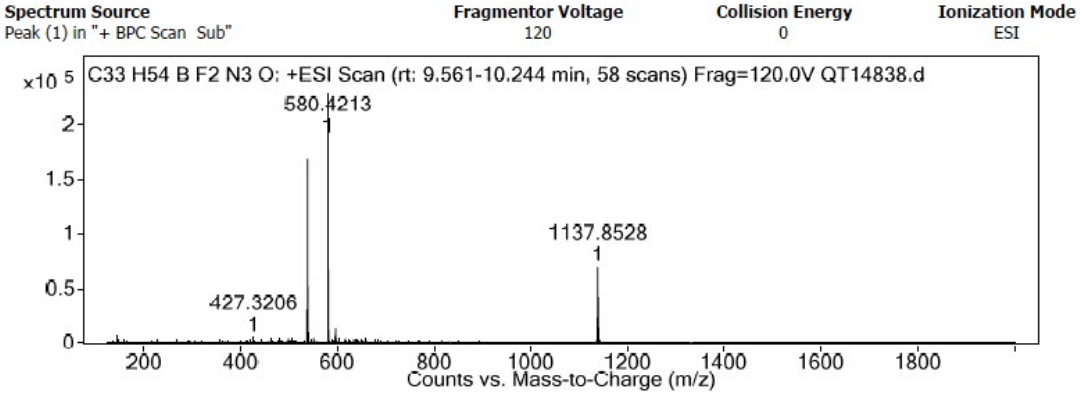
Supplementary information



^{1}H NMR spectrum of BDP-2C_8



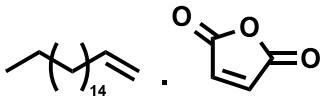
^{13}C NMR spectrum of BDP-2C_8



HRMS spectrum of BDP-2C_8

- Synthesis of amphiphilic polymer

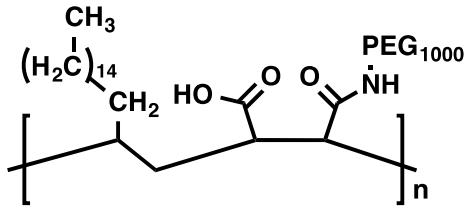
PMAO is sold with an average M_n of 30,000-50,000, which makes a polymer with ~100-140 repeating units per polymer molecule: M_A is the molecular weight of the repeating unit A.



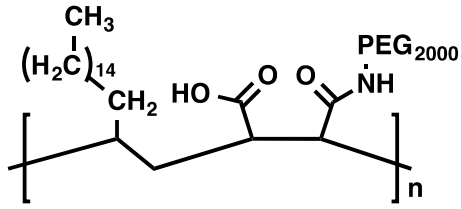
A
Repeating unit of PMAO
M= 350 g. mol^{-1}

For the synthesis, PMAO was considered as a single molecule of the repeating unit A with a molecular weight of 350 $g.mol^{-1}$.

Typical procedure for Half PEGylated and non-capped polymers (HP1, HP2)

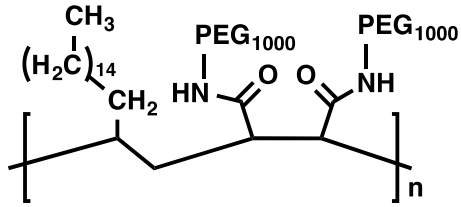


HP1. To a solution of PMAO (30 mg, 85.71 μmol) in degassed anhydrous DMF (3 mL) was added a solution of a Rhodamine-NH_2 (8.57 mol, 69 μL C=10 mg/mL in DMF) followed by DIEA (0.85 mmol, 200 mL, 10 eq) and a solution of Jeffamine-1000 (1 mL, C=100 mg/mL in DMF, 0.102 mmol, 1.2 eq). The reaction mixture was allowed to stir for 2 h before the addition of few drops of water. The solvents were evaporated. The crude was purified by size exclusion column using DCM/MeOH (1/1). 61 mg of HP1 was obtained as a pink sirup (Yield= 53%).

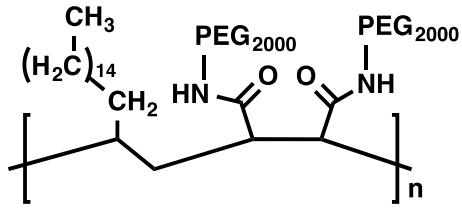


HP2. Using Jefamine-2000. Pink syrup, 53 mg, yield = 81%.

Typical procedure for fully PEGylated polymers (FP1, FP2)

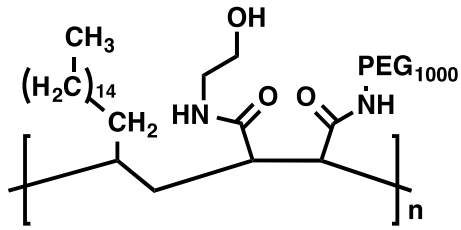


FP1. To a solution of PMAO (10 mg, 28.0 μmol) in degassed anhydrous DMF (3 mL) was added a solution of a Rhodamine-NH_2 (280 nmol, 23 μL C=10 mg/mL in DMF, 0.01 eq) followed by DIEA (280 μmol, 50 μL, 10 eq) and a solution of Jeffamine-1000 (870 μL, C=100 mg/mL in DMF, 0.102 mmol, 3 eq). The reaction mixture was allowed to stir for 2 h before the addition of HATU (21 mg, 54.0 μmol, 2 eq). The reaction was allowed to stir at 60°C overnight. The solvents were evaporated. The crude was purified by size exclusion column using DCM/MeOH (1/1). Pink syrup, 25 mg, yield = 38%.



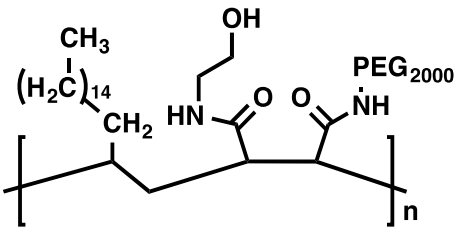
FP2. Using Jefamine-2000. Pink sirup, 85 mg, yield = 70%.

Typical procedure for ethanolamine-capped polymers (HP1c, HP2c)



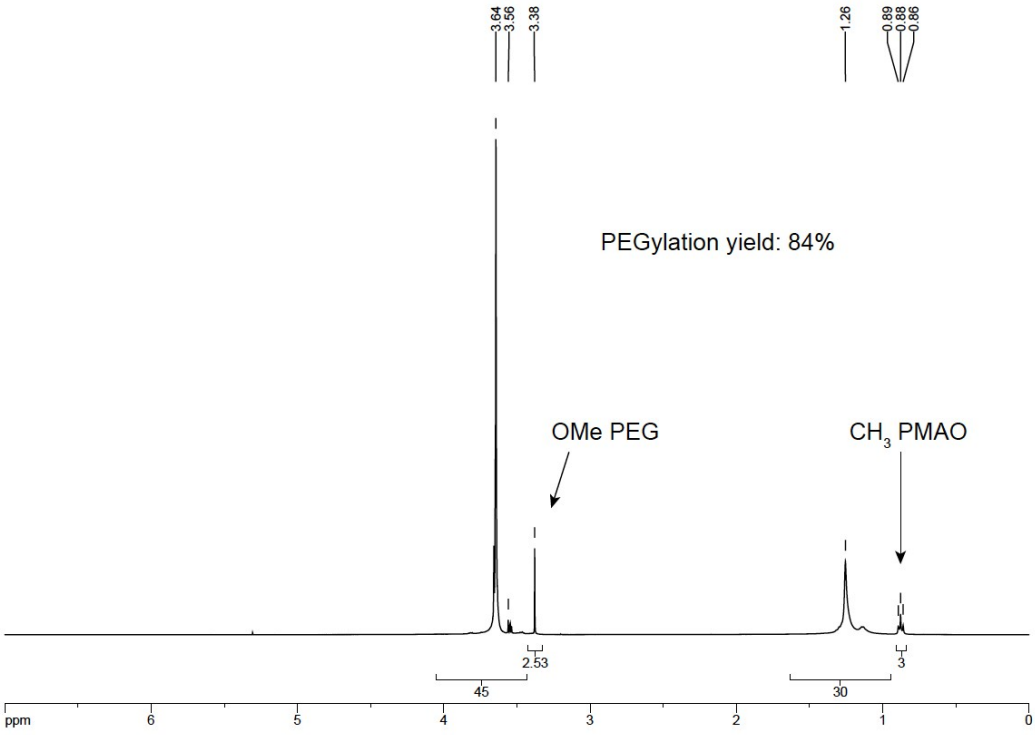
HP1c. To a solution of PMAO (30 mg, 85.0 μmol) in degassed anhydrous DMF (3 mL) was

added a solution of a Rhodamine-NH_2 (850 nmol, 69 µL C=10 mg/mL in DMF, 0.01 eq) followed by DIEA (850 µmol, 200 µL, 10 eq) and a solution of Jeffamine-1000 (860 µL, C=100 mg/mL in DMF, 0.102 mmol, 1 eq). The reaction mixture was allowed to stir for 2 h before the addition of ethanolamine (1.05 mL, 2 eq, 0.172 µmol, C= 10 mg/mL) and HATU (66 mg, 172.0 µmol, 2 eq). The reaction was allowed to stir at 60°C overnight. The solvents were evaporated. The crude was purified by size exclusion column using DCM/MeOH (1/1). Pink sirup, 57 mg, yield = 48%.

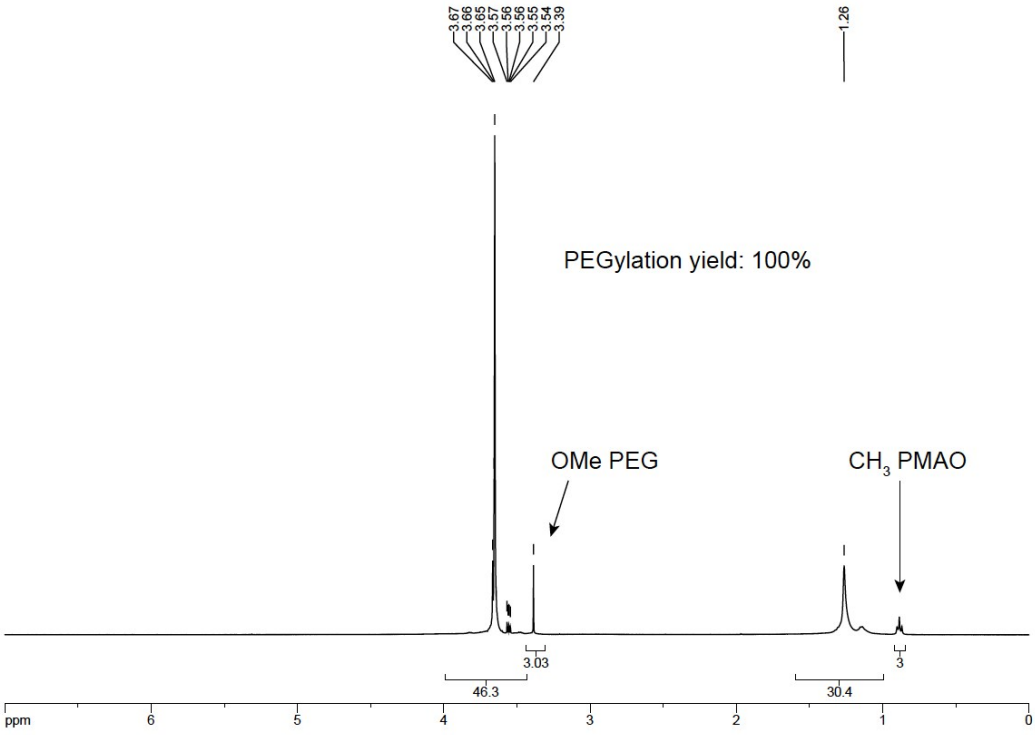


HP2c. Using Jefamine-2000. Pink sirup, 27 mg, yield = 40%.

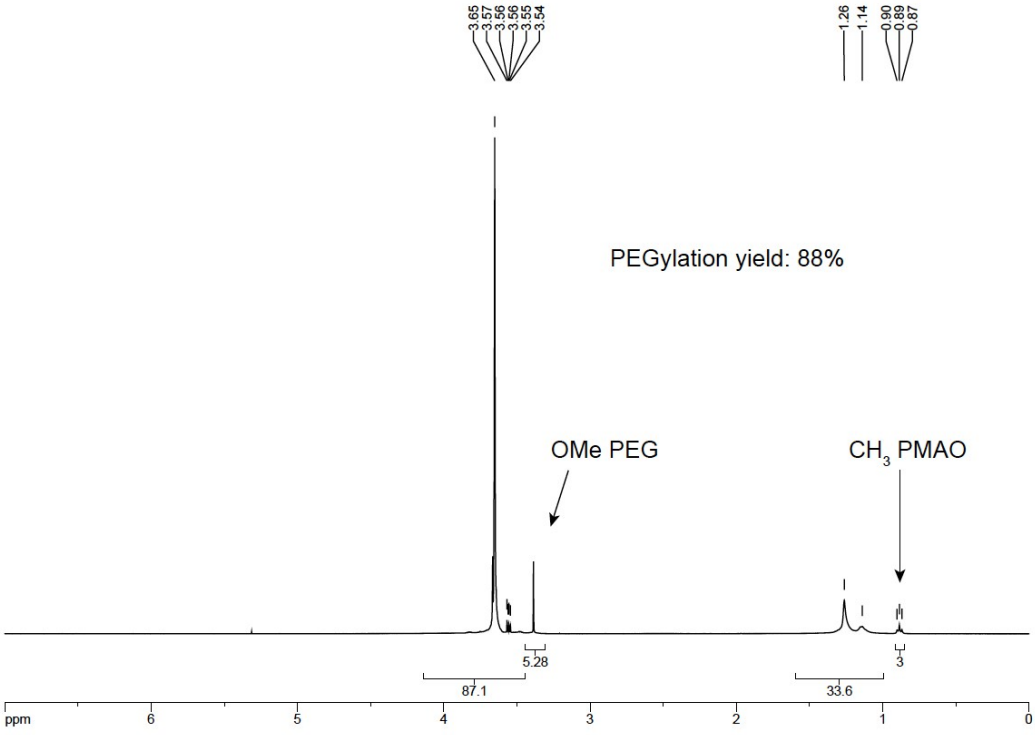
The composition of the polymers was confirmed and characterized using ^{1}H NMR. The PEGylation yield was determined by dividing the integration value of the signal of the terminal methoxy group (OMe) of the PEG at 3.3 ppm with the CH_3 signal of the hydrocarbon chain at 0.8 ppm. The PEGylation yields were all above 80%.



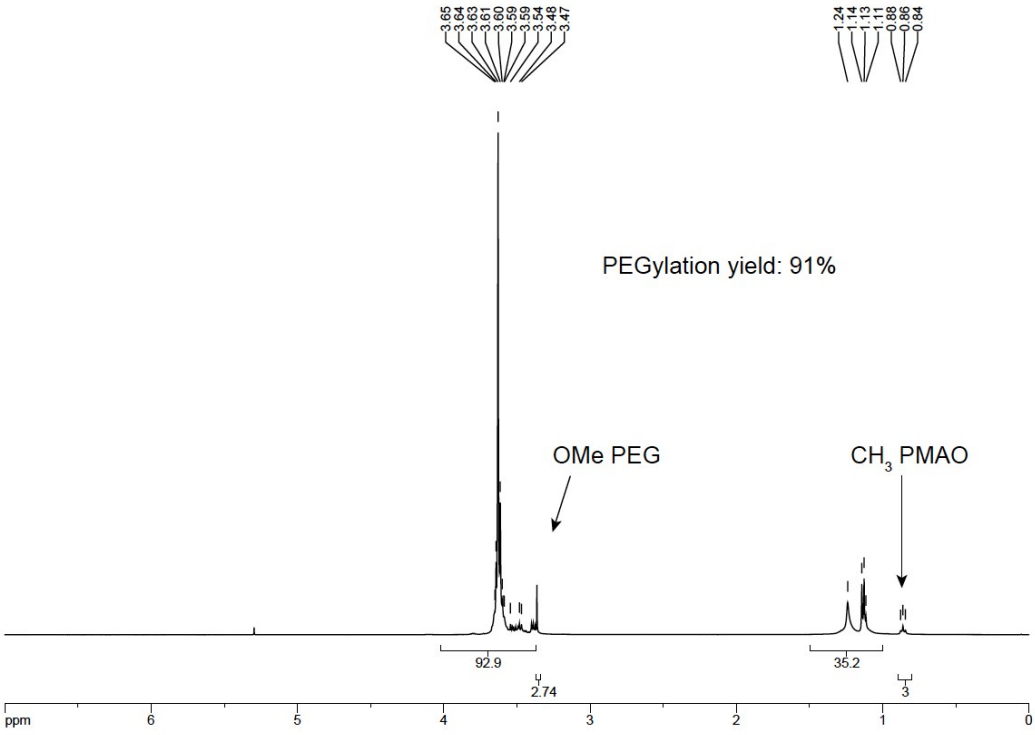
^{1}H NMR spectra of HP1 ($CDCl_3$, 400 MHz)



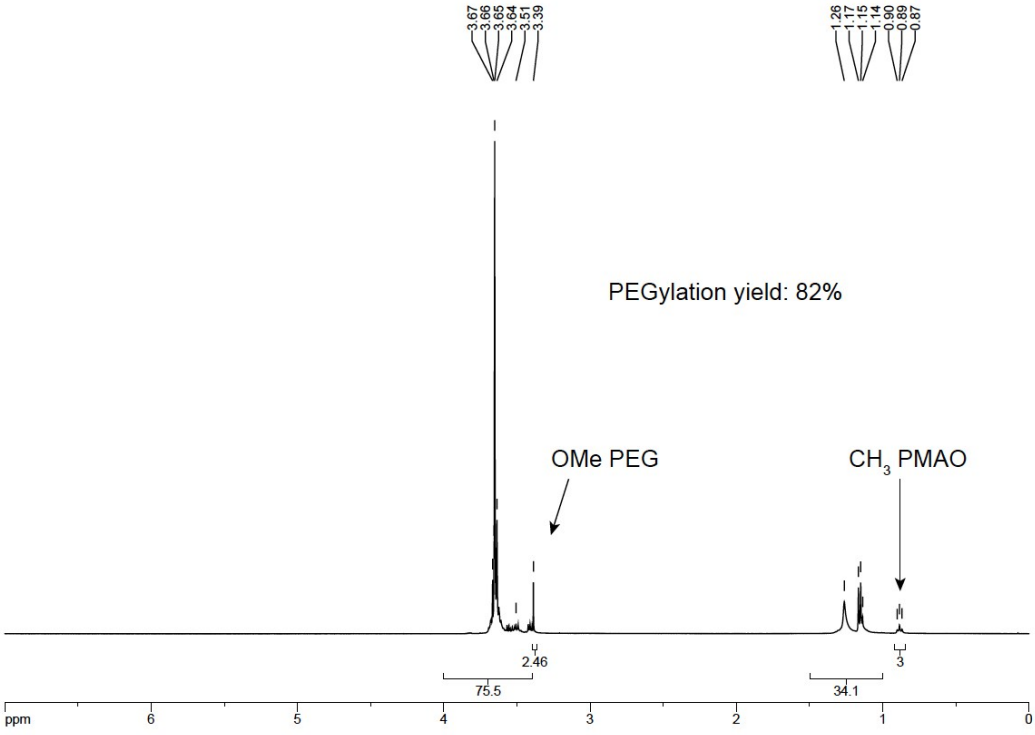
^{1}H NMR spectra of HP1c ($CDCl_3$, 400 MHz)



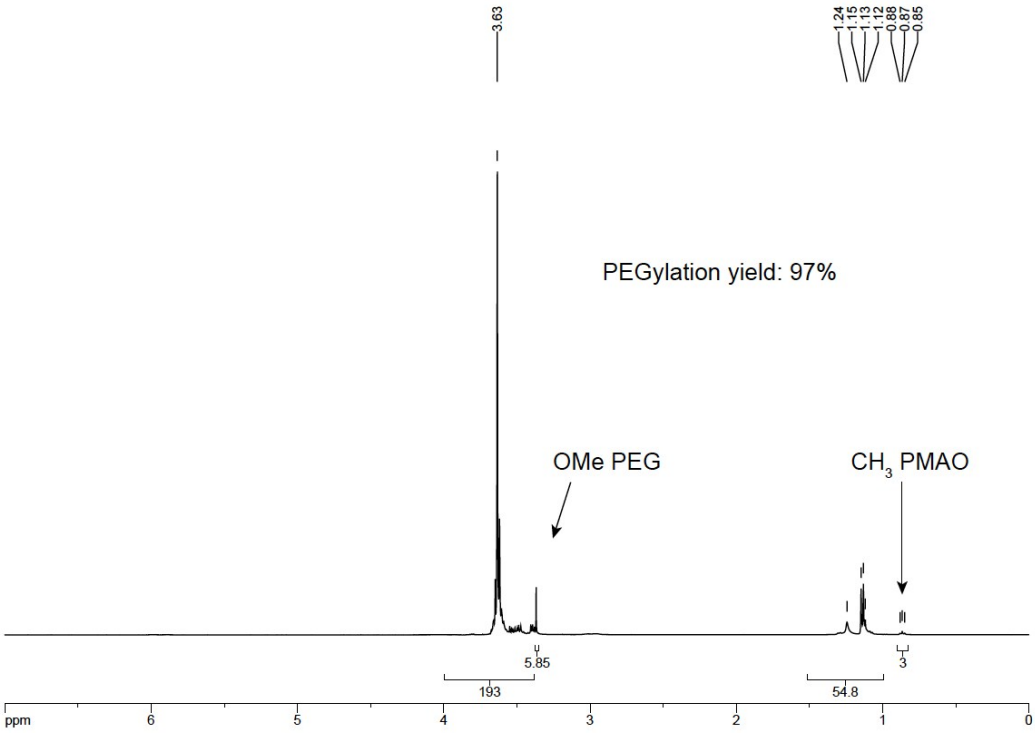
^{1}H NMR spectra of FP1 ($CDCl_3$, 400 MHz)



^{1}H NMR spectra of HP2 ($CDCl_3$, 400 MHz)



^{1}H NMR spectra of HP2c ($CDCl_3$, 400 MHz)



^{1}H NMR spectra of FP2 ($CDCl_3$, 400 MHz)

Spectroscopic studies of BDP-2C$_8$

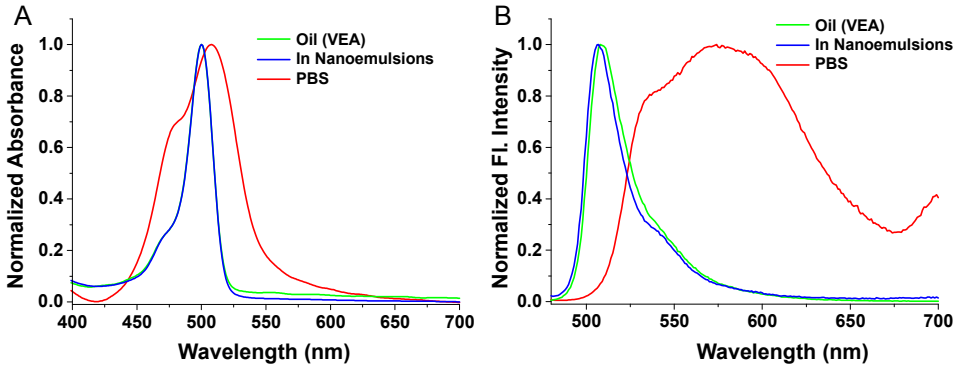


Figure S1. Normalized absorption (A) and emission (B) spectra of BDP-2C$_8$ at 1 μM in various media. In PBS (red lines), the broadening of the spectra and the red shift in emission are typical signs of aggregation. Excitation wavelength was 470 nm.

Table S1. Photophysical properties of BDP-2C_8.

	λABS_{max} (nm)	ε ($M^{-1}.cm^{-1}$)	FWHM[b] (nm)	λEm_{max} (nm)	FWHM[b] (nm)	ϕ[c]
VEA	500	93,000	23	507	24	0.82
NE[a]	501	86,300	23	510	29	0.93
PBS	508	31,100	63	577	113	0.19

[a] Nanoemulsions (44 nm) obtained by spontaneous emulsification in PBS of 1 wt % BDP-2C_8 loaded-VEA in the presence of 50 wt % Kolliphor ELP® as surfactant.
[b] FWHM is the Full Width at Half maximum of the peak, this value depicts the broadness of the peak.
[c] Fluorescein (in 0.1 M NaOH in water) was used as reference.

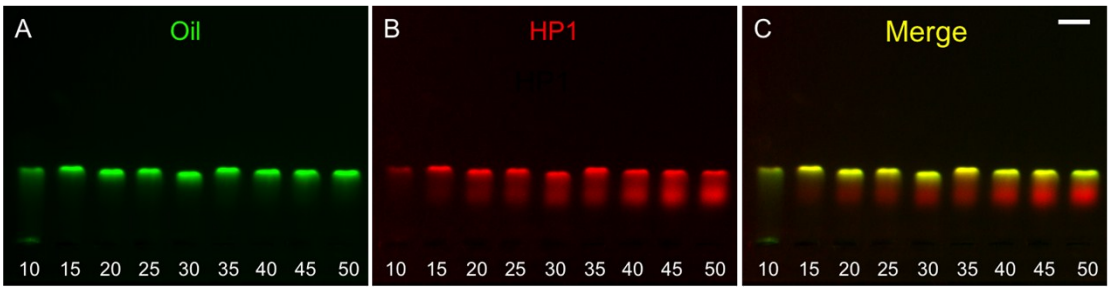


Figure S2. Electrophoresis revealed in the green channel (A), red channel (B) and the merged channel (C) of formulation obtained with various polymer/oil wt %. The concentration of oil (containing 1 wt % BDP-2C_8) was fixed at 80 µg/mL and the amount of HP1 was increased according to the indicated HP1 wt %. Scale bar is 0.5 cm.

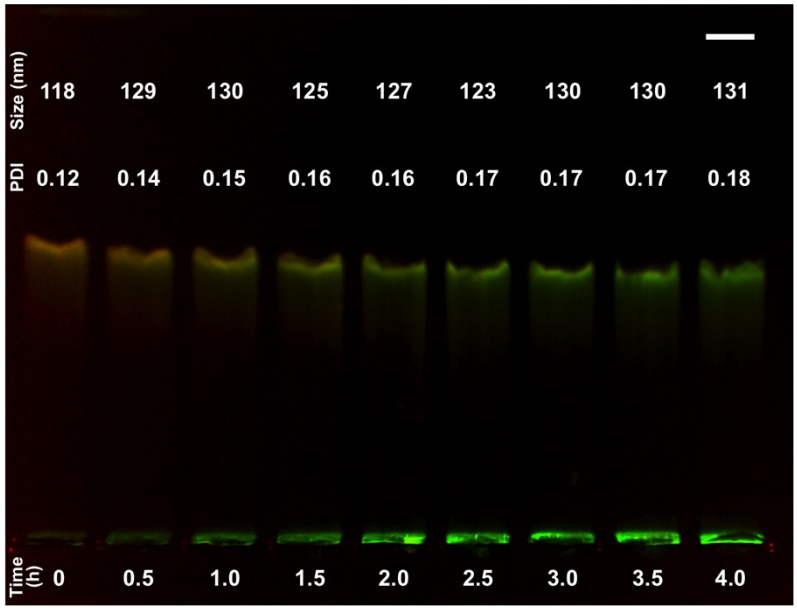


Figure S3. Stability of HNPs (20 wt %) over 4 h at 60°C. The figure features the merged green and red channels of the electrophoresis image as well as the size and PDI measured by DLS for each time points. Scale bar is 0.5 cm.

Table S2. Measured ζ potential of PMAO-based amphiphilic polymers formulated at 0.02 μg/mL in distilled water. Conductivity was at 0.05 mS/cm.

Polymer	HP1	HP2	HP1c	HP2c	FP1	FP2
ζ (mV)	-24	-29	-26	-21	-28	-28

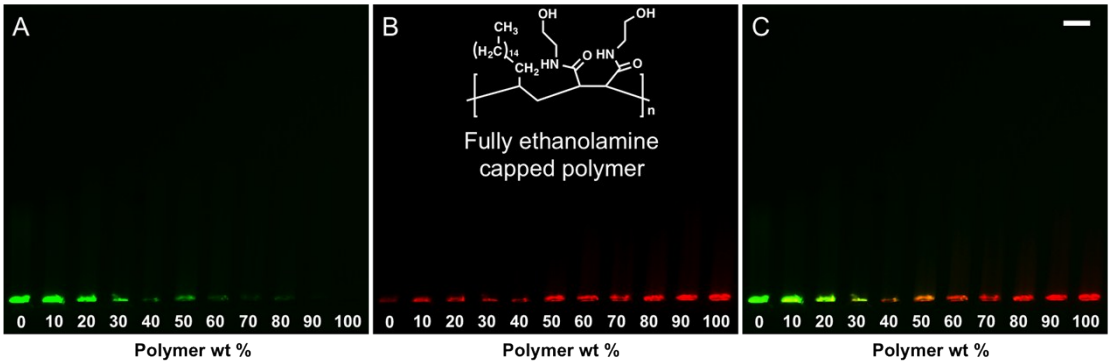


Figure S4. Electrophoresis revealed in the green channel (A), red channel (B) and the merged channel (C) of formulation obtained with various fully ethanolamine-capped polymer/oil wt %. Scale bar is 0.5 cm.

References

(1) Trofymchuk, K.; Valanciunaite, J.; Andreiuk, B.; Reisch, A.; Collot, M.; Klymchenko, A. S. BODIPY-Loaded Polymer Nanoparticles: Chemical Structure of Cargo Defines Leakage from Nanocarrier in Living Cells. *J. Mater. Chem. B* **2019**, *7* (34), 5199–5210. https://doi.org/10.1039/C8TB02781A.

(2) Dukhno, O.; Przybilla, F.; Collot, M.; Klymchenko, A.; Pivovarenko, V.; Buchner, M.; Muhr, V.; Hirsch, T.; Mély, Y. Quantitative Assessment of Energy Transfer in Upconverting Nanoparticles Grafted with Organic Dyes. *Nanoscale* **2017**, *9* (33), 11994–12004. https://doi.org/10.1039/C6NR09706E.