# Supporting Information

for

# Systematic study of perfluorocarbon nanoemulsions stabilized by polymer amphiphiles

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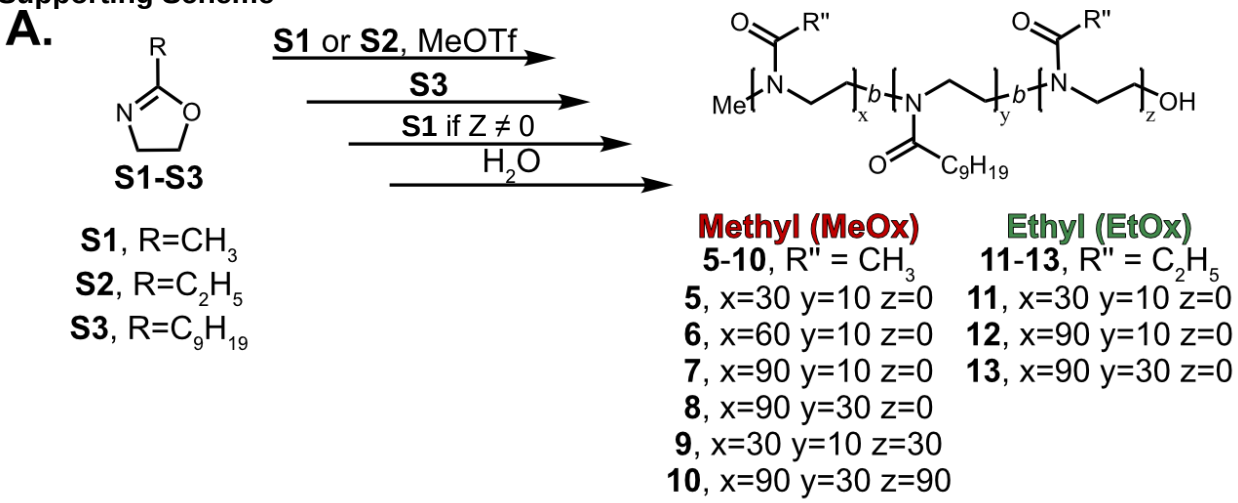
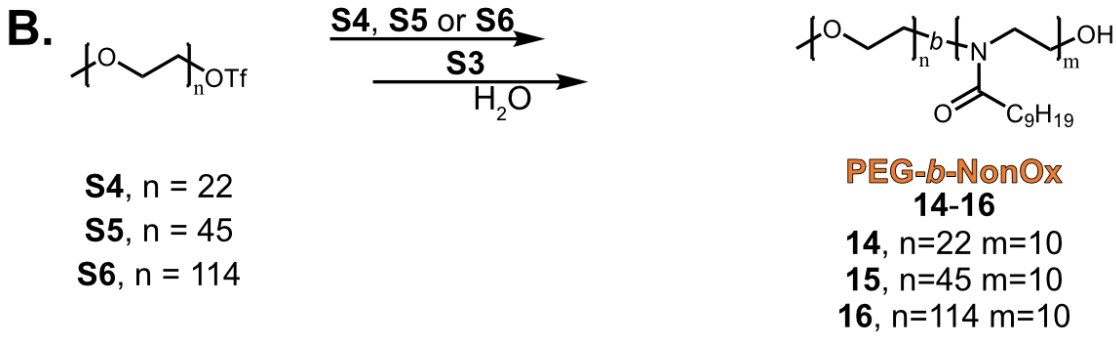
**Supporting Scheme**




**Scheme S1**.
(A) Synthetic scheme for POx containing amphiphiles. Monomers **S1** (MeOx), or **S2** (EtOx) were added to a flame dried microwave vial and dissolved in acetonitrile (MeCN). The initiator, methyl triflate (MeOTf), was added and the mixture was heated at 140 °C in the microwave. The block lengths were tuned by initiator to monitor ratio and reaction time. After completion of the first block, **S3** (NonOx) was added and reacted further at 140 °C. The reaction was quenched with excess MilliQ water. All polymers were purified by dialysis against a dichloromethane: methanol mixture overnight. Block lengths have been rounded for simplicity, exact block lengths are found in the polymer characterization section.
(B) Synthetic scheme for $PEG_n$-*b*-$NonOx_{10}$ amphiphiles. Monomer **S3** (NonOx) was added to a flame dried microwave vial and dissolved in MeCN. After brief mixing, the desired $PEG_n$-tosylate (**S4**–**S6**) was added and reacted at 140 °C in the microwave. Once complete, the reaction was quenched with excess MilliQ water and purified by washing with water and dialysis against MeOH.

**Supporting Figures**

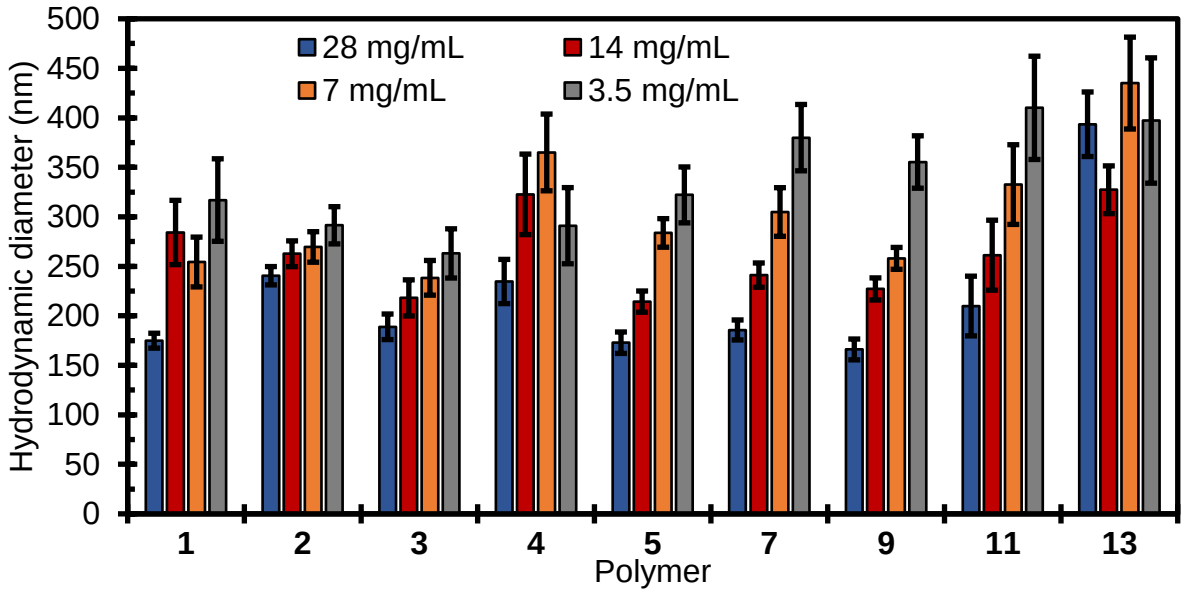



**Figure S1**. Size and dispersity of PFC nanoemulsions formed with various concentrations of polymer surfactants **1** (PF68)**, 2** (PF127), **3** (Zonyl FSO), **4** (Zonyl FSN), **: 5** [P(MeOx$_{30}$-*b*-NonOx$_{10}$)], **7** [P(MeOx$_{90}$-*b*-NonOx$_{10}$)], **9** [P(MeOx$_{30}$-*b*-NonOx$_{10}$-*b*-MeOx$_{30}$)], **11** [P(EtOx$_{30}$-*b*-NonOx$_{10}$)], **13** [P(EtOx$_{90}$-*b*-NonOx$_{10}$)].

PFC nanoemulsions were formed following the **general nanoemulsion procedure** purposefully varying the concentration of surfactant from 28 mg/mL (2.8 wt%) to 3.5 mg/mL (0.35 wt%). After formulation, emulsions were analyzed following the **nanoemulsion size analysis**. Bars represent the average of three independent samples. Error bars represent the product of the $z_{average}$ and the polydispersity (PDI) of three independent samples.

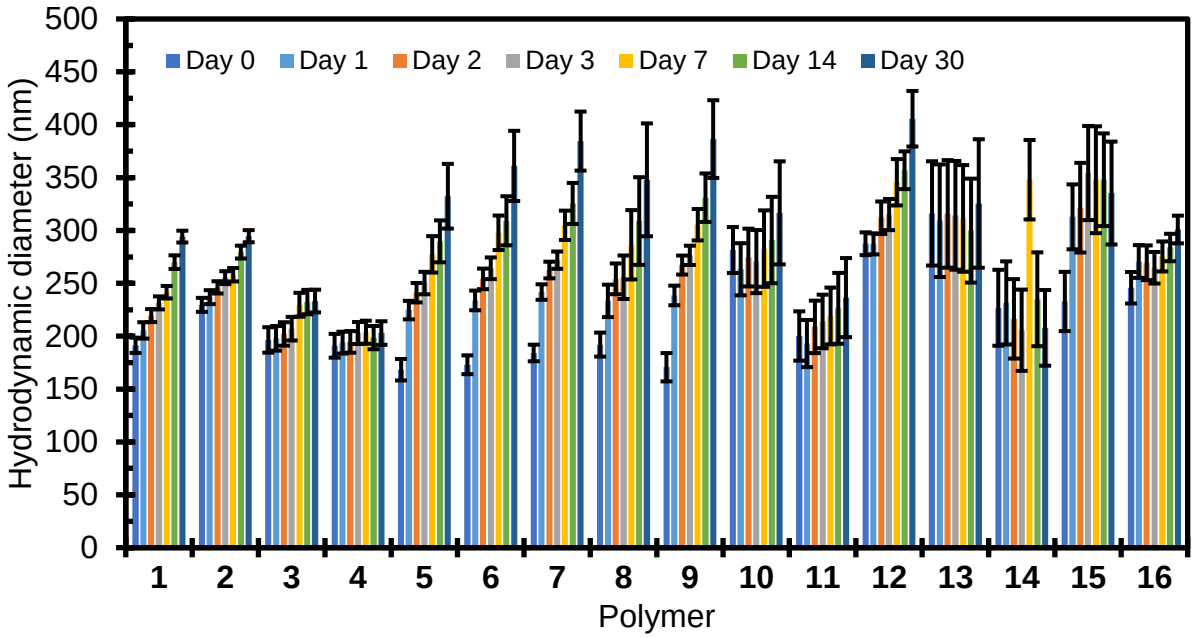


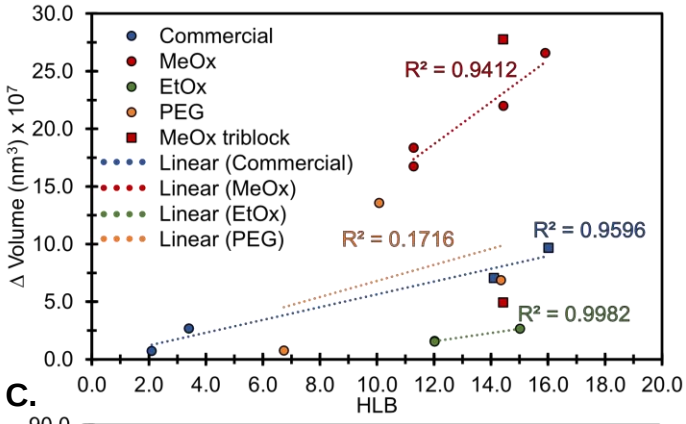

**Figure S2**. Stability of PFC nanoemulsions stabilized by polymer surfactants **1–16** over 30 days.

PFC nanoemulsions were prepared following the **general nanoemulsion procedure**. On Day 0, 1, 2, 3, 7, 14, and 30 PFC nanoemulsions were resuspended via vortexing and pipetting vigorously. The hydrodynamic diameter and polydispersity (PDI) were monitored following the **general nanoemulsion analysis**. The bars represent the average of three independent samples. Error bars represent the product of the $z_{average}$ and the PDI of three independent samples.

**A.**

| Polymer | Hydrophilic lipophilic balance (HLB) | Δ Volume (30 day) | Leaching % (14 day) |
|---|---|---|---|
| **1** | 16.0 | 9.7 | 6.1 |
| **2** | 14.1 | 7.0 | 3.1 |
| **3** | 3.4 | 2.7 | 59.3 |
| **4** | 2.1 | 0.7 | 43.3 |
| **5** | 11.3 | 16.8 | 14.1 |
| **6** | 14.4 | 21.9 | 11.9 |
| **7** | 15.9 | 26.6 | 15.4 |
| **8** | 11.3 | 18.4 | 14.5 |
| **9** | 14.4 | 27.8 | 12.4 |
| **10** | 14.4 | 4.9 | 20.8 |
| **11** | 12.0 | 1.6 | 71.9 |
| **12** | 15.0 | 2.7 | 56.1 |
| **13** | 12.0 | 1.5 | 67.2 |
| **14** | 6.7 | 0.8 | 78.0 |
| **15** | 10.1 | 13.6 | 67.4 |
| **16** | 14.3 | 6.9 | 35.0 |

**B.**




**C.**

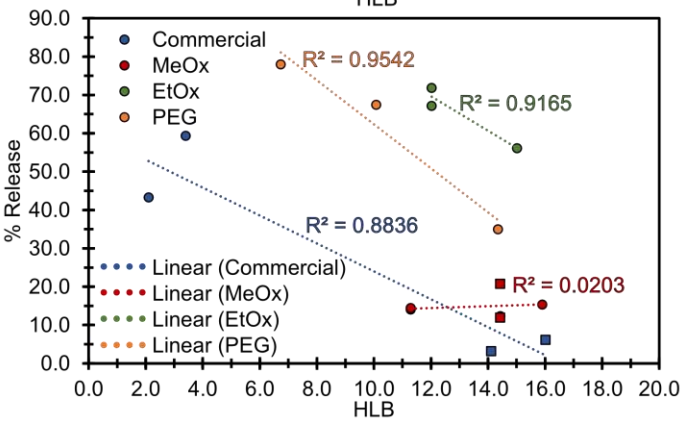



**Figure S3**. Hydrophilic-lipophilic balance (HLB) ratio of polymers **1**–**16** in relation to the change in volume of PFC emulsions over 30 days and leaching after 14 days.

A. HLB calculations determined by Griffin's non-ionic method:[1,2]
HLB is defined as $\mathrm{HLB} = \frac{\mathrm{E}}{5}$ for poly(oxyethylene)ester containing polymers where E is defined as the mass (or weight) percent of oxyethylene that can be more precisely calculated by $\mathrm{E} = \frac{\mathrm{M_{PEG}}}{\mathrm{M_{Total}}} \mathrm{x}\, 100$.

Sample calculation (PF-68, **1**):
$\mathrm{E} = \frac{76n*44}{8350}$
where 76 is the length of the PEG unit, n = 2 because PF-68 is a triblock copolymer, and 44 is the molecular weight of PEG.
$\mathrm{HLB} = \frac{\mathrm{E}}{5}$
The same calculations were performed for the POx containing polymers.[3,4]
Zonyl FSO and Zonyl FSN HLB were taken from patent[5] "Dry cleaning system with low HLB surfactant" (US6461387B1).

B. HLB ratios plotted against the change in volume of PFC nanoemulsions over 30 days. Raw data are seen in Figure 2 and Figure S2. Squares represent tri-block copolymers and circles represent diblock copolymers. Commercial polymers and respective trendline are found in Blue, P(MeOx) and trendline in red, P(EtOx) and trendline in green, and PEG-NonOx and trendline in orange. Note: P(MeOx) triblocks (**9**, **10**) are not included in the P(MeOx) trendline.
C. HLB ratios plotted against the % release of fluorous coumarin **17**. Raw data are found in Figure 3. Squares represent tri-block copolymers and circles represent diblock copolymers. Commercial polymers and respective trendline are found in Blue, P(MeOx) and trendline in red, P(EtOx) and trendline in green, and PEG-NonOx and trendline in orange.


(1) Griffin, W.C. Classification of surface-active agents by HLB. *J. Soc. Cosmet. Chem.* **1949**, *1*, 311-326.
(2) Pasquali, R. C.; Taurozzi, M. P.; Bregni, C. Some considerations about the hydrophilic-lipophilic balance system. *Int. J. Pharm.* **2008**, *356*. 44-51.
(3) Delage, B.; Brious, B.; Brossier, T.; Catrouillet, S.; Robin, J.J.; Lapinte, V. Polyoxazoline associated with cardanol for bio-based linear alkyl benzene surfactants. *Polym. Int.* **2019**, *68*, 755-763.
(4) Giardi, C.; Lapinte, V.; Charnay, C.; Robin, J. J. Nonionic polyoxazoline surfactants based on renewable source: Synthesis, surface and bulk properties. *Reactive and Functional Polymers, Elsevier*, **2009**, *69*, 643-649.
(5) Jureller, S.H.; Kerschner, J. L.; Murphy, D. S. **2002**, *Dry cleaning system with low HLB surfactant*, US6461387B1.

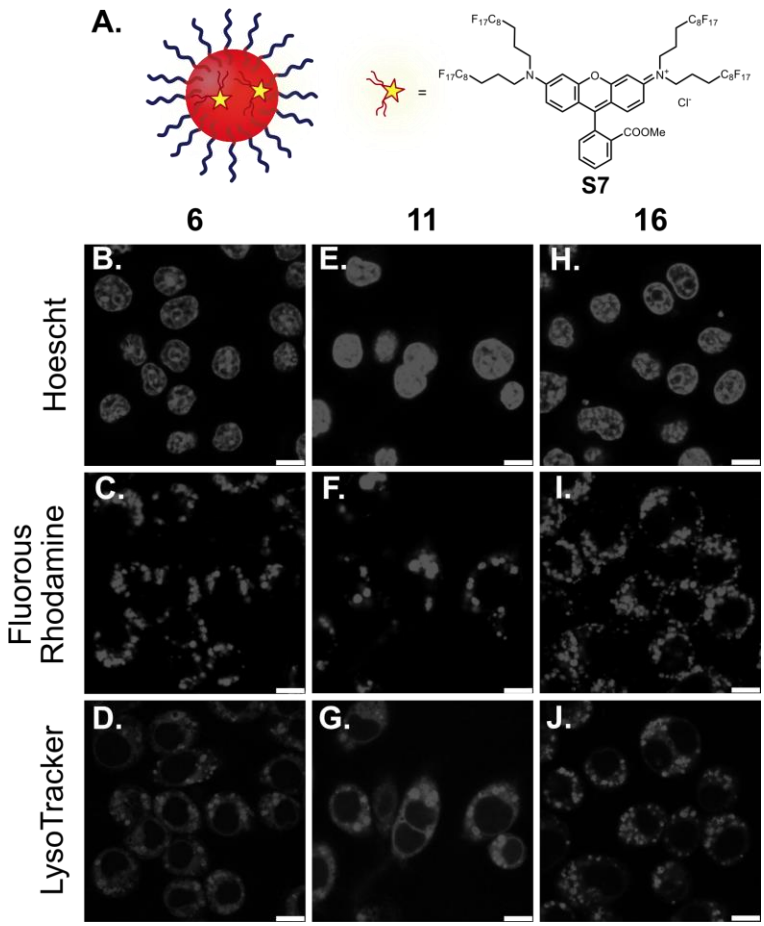



**Figure S4.** Single channel images for Figure 4A.

(A) PFC Nanoemulsions containing fluorous rhodamine (**S7**).[6] (B-J) Single channel images. Hoescht dye fluorescence (B, E, H), fluorous rhodamine fluorescence (C, F, I), LysoTracker green (D, G, J). In all cases, cells were treated with Hoescht stain and LysoTracker before imaging. Scale bars represent 7.5 μm.

(6) Sletten, E. M.; Swager, T. M. Fluorofluorophores: Fluorescent Fluorous Chemical Tools Spanning the Visible Spectrum. *J. Am. Chem. Soc.* **2014**, *136*, 13574–13577.

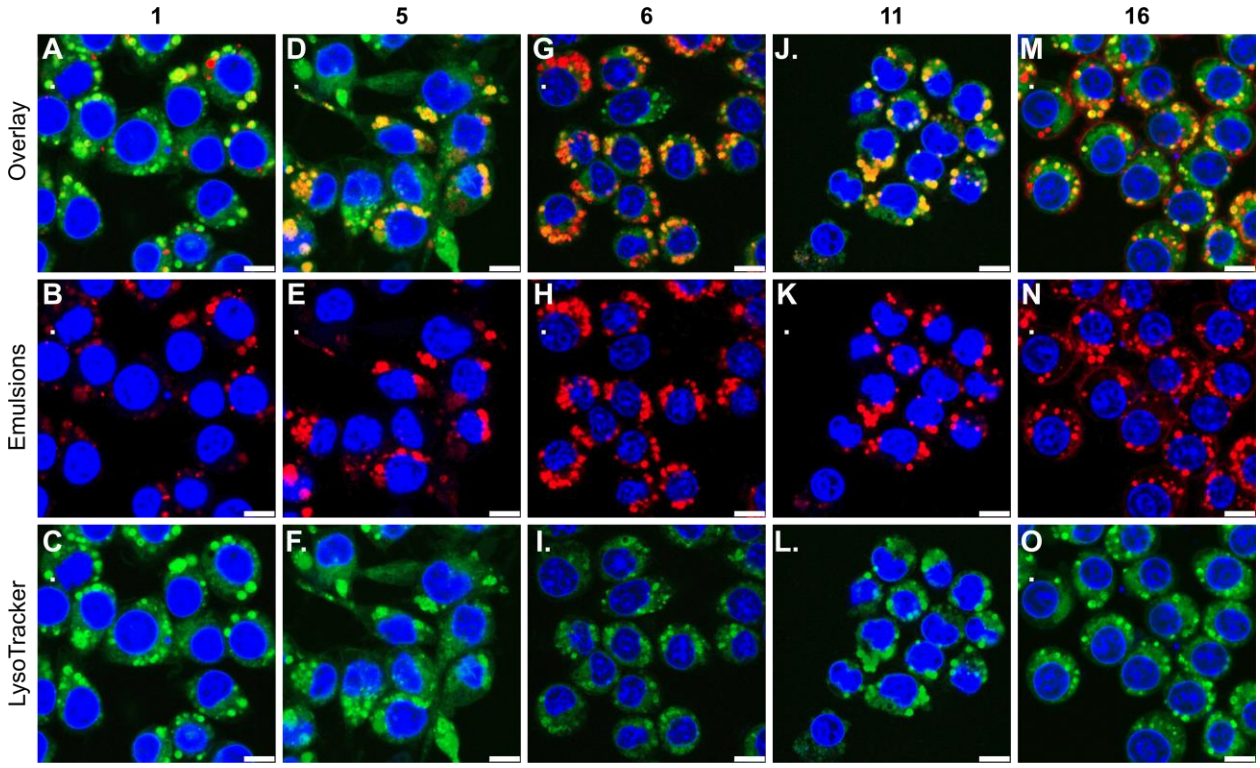



**Figure S5.** Colocalization of PFC nanoemulsions stabilized by polymer surfactants (**1** (PF68), **5** [P($MeOx_{30}$-*b*-$NonOx_{10}$)], **6** [P($MeOx_{60}$-*b*-$NonOx_{10}$)], **11** [P($EtOx_{30}$-*b*-$NonOx_{10}$)], **16** $PEG_{5K}$-*b*-$NonOx_{10}$) and lysosomes imaged via LysoTracker. Emulsions were loaded with fluorous rhodamine (**S7**, red – B, E, H, K, N) for visualization. Cells were stained with Hoescht (blue) and LysoTracker (green – C, F, I, L, O). Cells were imaged via excitation at 405 nm to visualize Hoescht, 488 nm to visualize the LysoTracker green (C, F, I, L, O), 532 nm to visualize the rhodamine (B, E, H, K, N), and merged to show colocalization (A, D, G, J, M). Scale bars represent 7.5 μm.

Perfluorocarbon nanoemulsions containing fluorous rhodamine were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine (**S7**) dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 3.7 μg) was added and the emulsions were resonicated.

RAW264.7 cells were treated with emulsions stabilized by polymer surfactants (**1**, **5**–**6**, **11**, **16**) in basal media for 3 hr at 37 ˚C, 5% $CO_2$. After treatment, cells were washed with media 3x, lithium chloride buffer (LiCl) buffer 3x (see general cell procedures for composition) and replaced with OptiMEM containing cellular stains. PFC emulsions are dense and settle onto the cells. Consequently, to remove the emulsions, slight rocking was necessary. Cells were stained with Hoechst and LysoTracker Green DND-26.

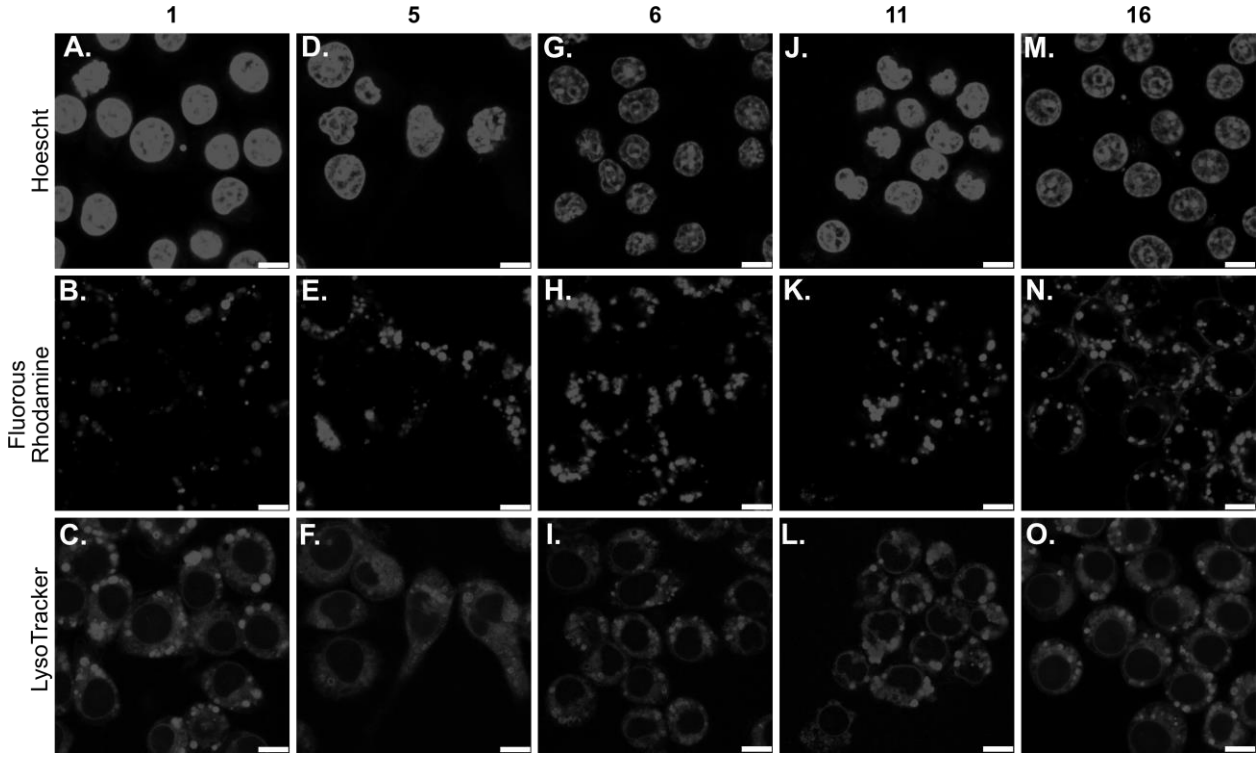



**Figure S6**. Single channel images for Figure S5.

Hoescht dye fluorescence (A, D, G, J, M), fluorous rhodamine (**S7**) fluorescence (B, E, H, K, N), LysoTracker green (C, F, I, L, O). In all cases, cells were treated with Hoescht stain and LysoTracker before imaging. Scale bars represent 7.5 μm.

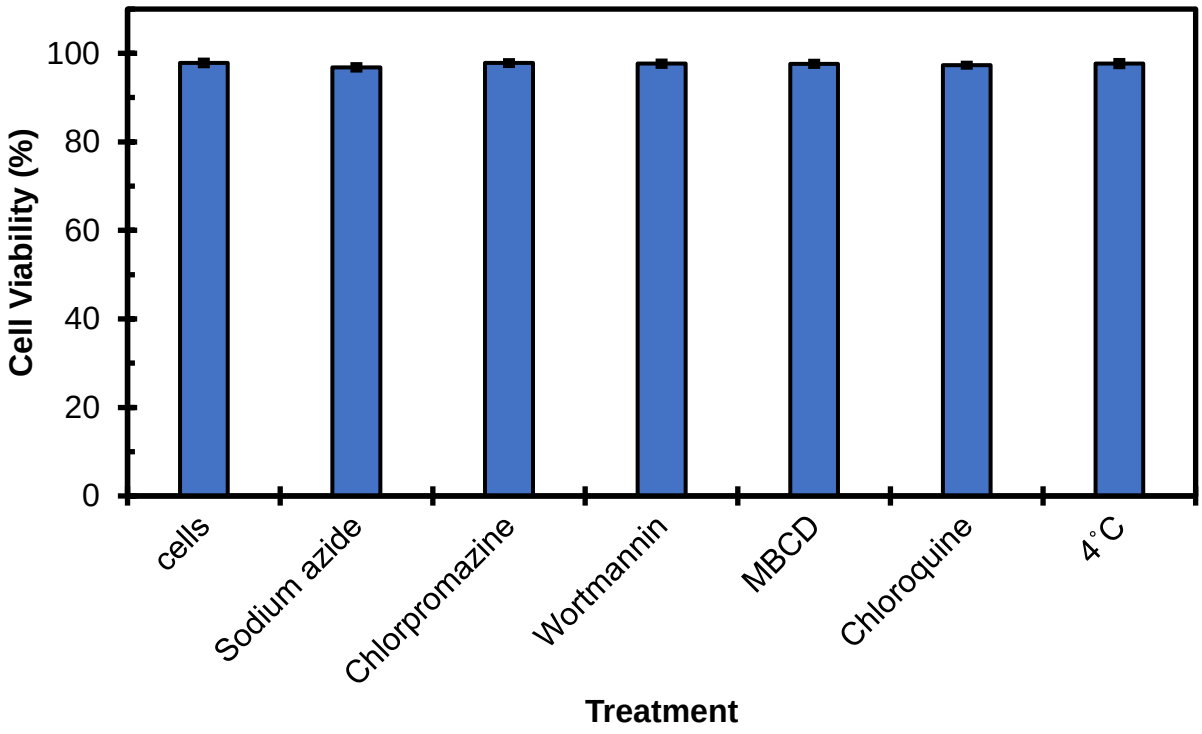



**Figure S7**. RAW264.7 cell viability after treatment with inhibitors $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM).

RAW264.7 cells were incubated with inhibitors in basal DMEM at concentrations indicated above for 4 h at 37 ˚C, 5% $CO_2$. Cells were placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media, LiCl buffer, PBS, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Cells were stained with propidium iodide (2 μl, 1 mg/mL, 15 min at 4 ºC). Cell viability was analyzed by the FL2 channel on a FACSCalibur flow cytometer with 15,000 cells collected per sample. Error bars represent the standard deviation of 3 replicate experiments.

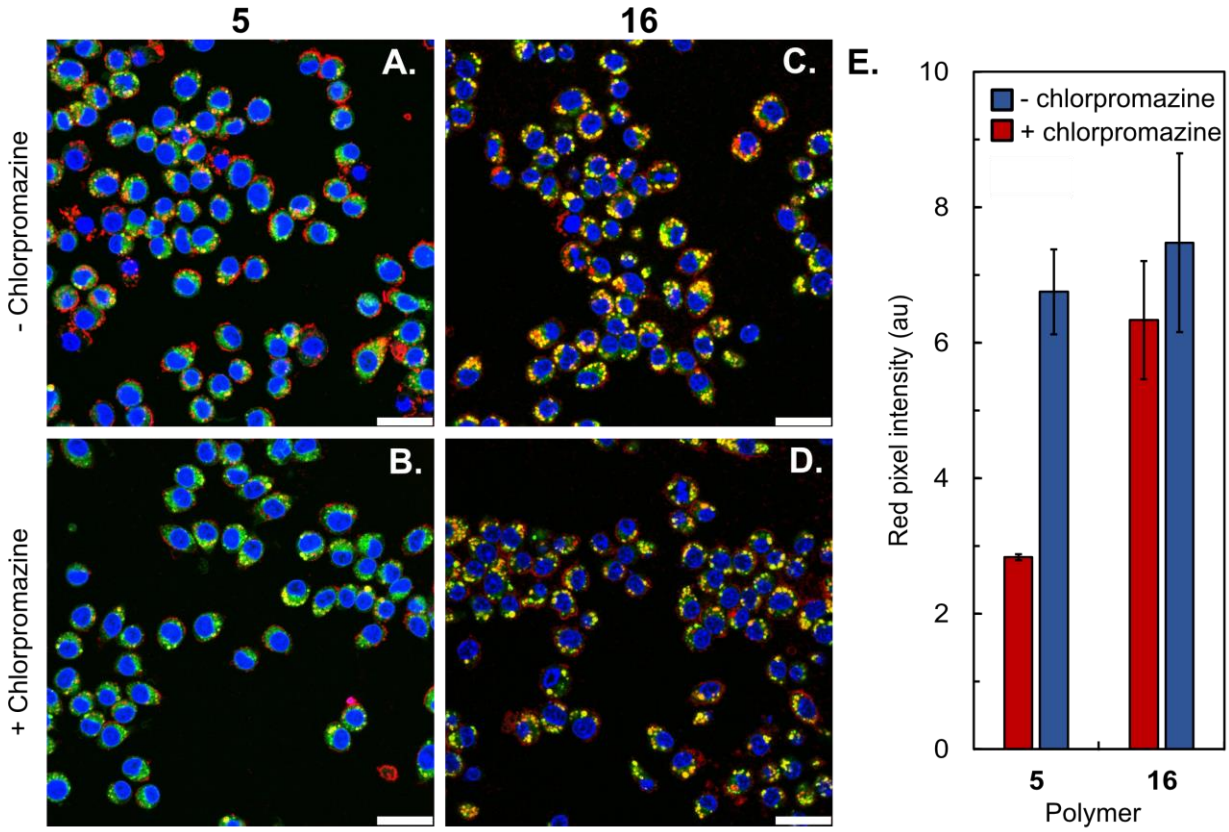



**Figure S8**. Uptake of PFC nanoemulsions stabilized by **5** [P($Me_{30}$-*b*-$Non_{10}$)] and **16** ($PEG_{5K}$-*b*-$NonOx_{10}$) in RAW264.7 cells in the presence or absence of chlorpromazine. (A–D) Uptake visualized by confocal microscopy and (E) quantification of fluorous rhodamine (**S7**) pixel intensity.

Perfluorocarbon nanoemulsions containing fluorous rhodamine (**S7**) were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine (**S7**) dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 3.7 μg) was added and the emulsions were resonicated.

RAW cells were either treated with emulsions (A, C) or emulsions and 60 μM chlorpromazine (B, D). Cells were incubated with chlorpromazine for 1 h before addition of emulsions and then further incubated for 3 h at 37 ˚C, 5% $CO_2$. After removal of excess emulsions, the cells were stained with Hoescht (blue), and LysoTracker (green). All channels (Hoescht, emulsions and LysoTracker) overlaid are seen in A–D. Scale bar represents 25 μm. (E) The pixel intensity of fluorous rhodamine (**S7**) fluorescence for each image was analyzed by ImageJ. Red bars are cells treated with chlorpromazine and blue bars are cells not treated with chlorpromazine. Bars represent the average of three independent images and error bars represent the standard deviation.

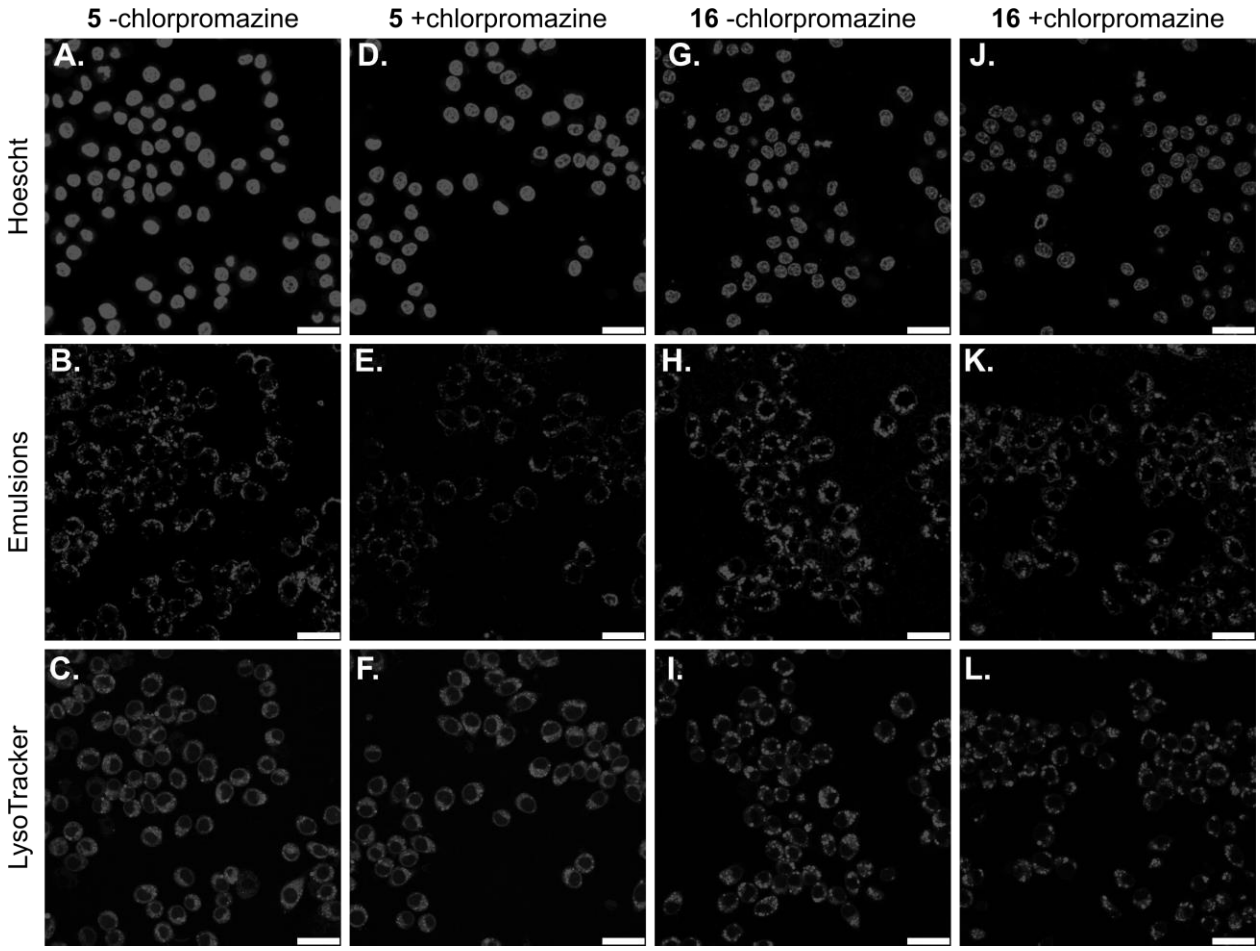



**Figure S9.** Single channel images for Figure S8.

Hoescht dye fluorescence (A, D, G, J), fluorous rhodamine fluorescence (B, E, H, K), LysoTracker green (C, F, I, L). In all cases, cells were treated with Hoescht stain and LysoTracker before imaging. Scale bars represent 25 μm.

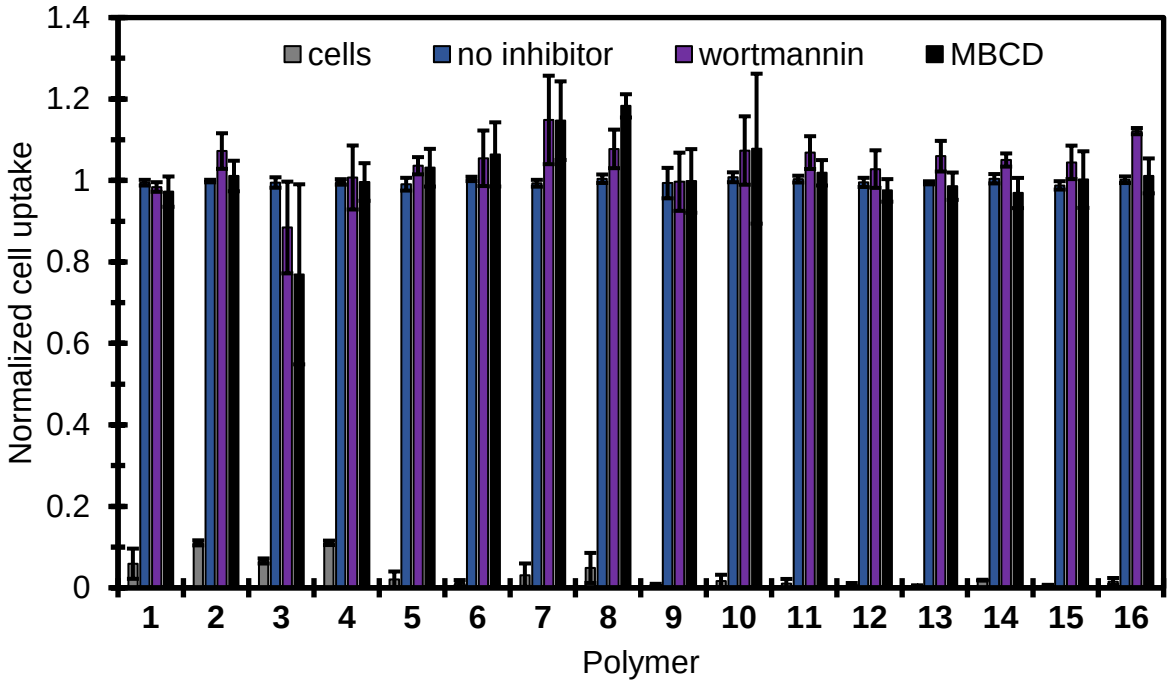
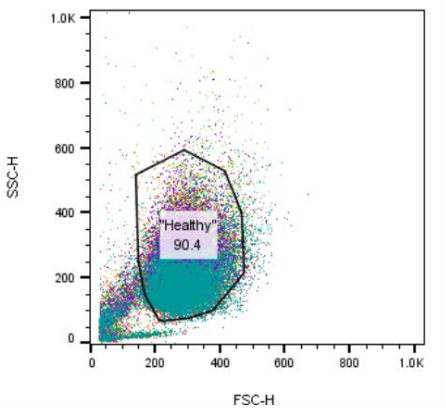



**Figure S10**. RAW264.7 cell uptake of PFC nanoemulsions stabilized by amphiphiles **1**–**16** after treatment with buffer (gray), emulsions but not inhibitors (blue), inhibitors Wortmannin (0.4 μM, purple) and MβCD (20 μM, black).

RAW264.7 cells were incubated with inhibitors in basal DMEM at concentrations indicated above for 1 h at 37 ˚C, 5% $CO_2$. Emulsions were added (10 μL) and incubated for a further 3 h at 37 ˚C, 5% $CO_2$. Cells were placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media, LiCl buffer, PBS, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer with 15,000 cells collected per sample. Data was normalized to cells treated with emulsions but not inhibitors (blue) for each polymer. Each bar is the average of three replicate experiments. Error bars represent the standard deviation of 3 replicate experiments.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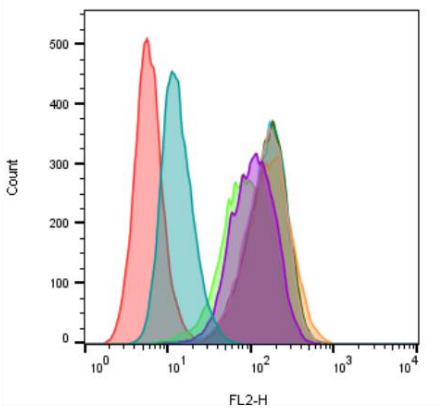
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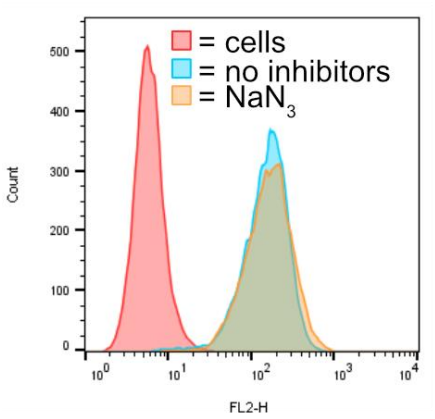
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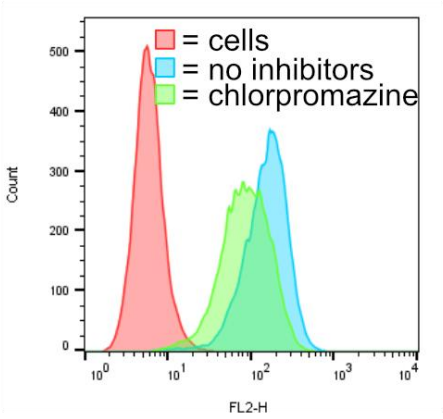
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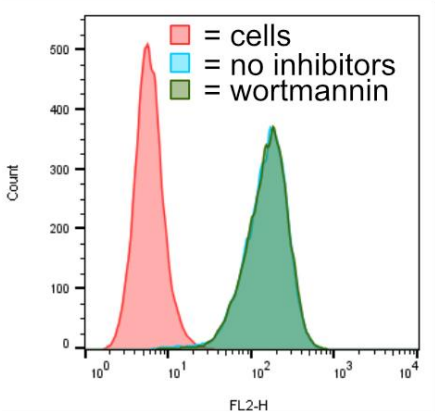
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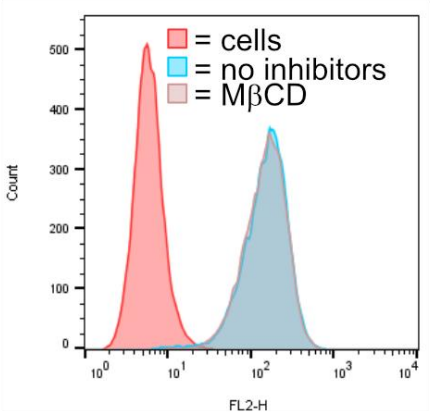
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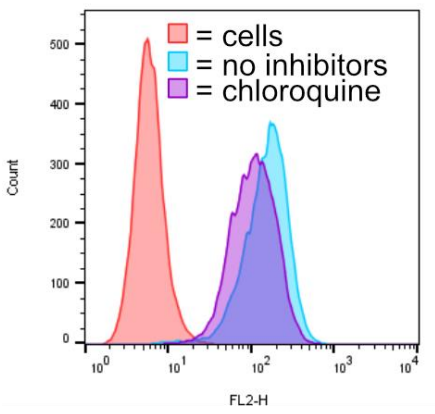
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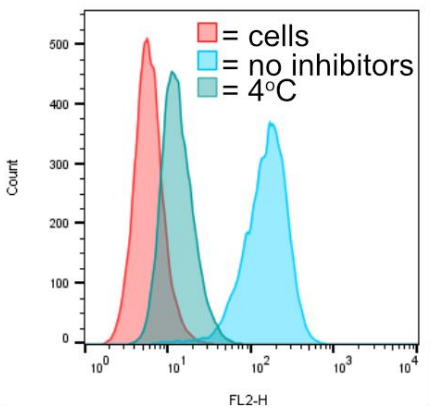
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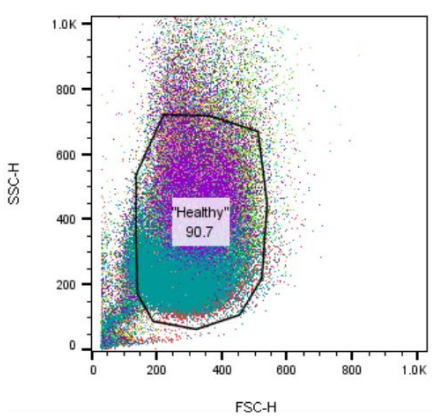
**Figure S11**. Histograms for flow cytometry data of Pluronic F-68 (**1**) in Figure 4C.

Perfluorocarbon nanoemulsions containing fluorous rhodamine (**S7**) were prepared by making nanoemulsions following the **general nanoemulsion formulation** procedure. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and the emulsions were resonicated.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate. The cells were allowed to adhere overnight. The media was replaced with basal DMEM and inhibitors: $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM) and incubated for 1 h (37 ˚C, 5% $CO_2$). Emulsions were added (10 μL) and incubated for a further 3 h (37 ˚C, 5% $CO_2$). For 4 ˚C, emulsions were added to cells and placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer with 15,000 cells were collected per sample.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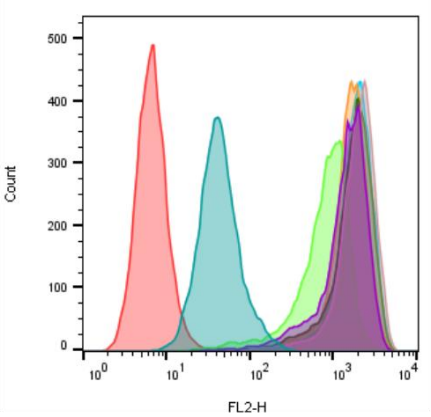
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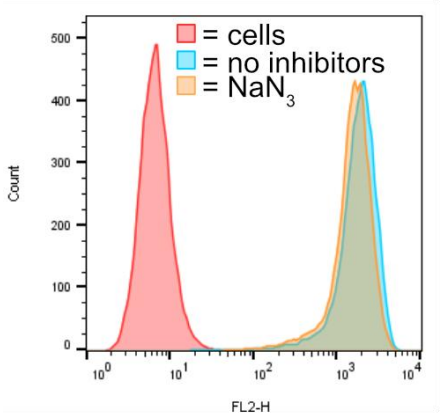
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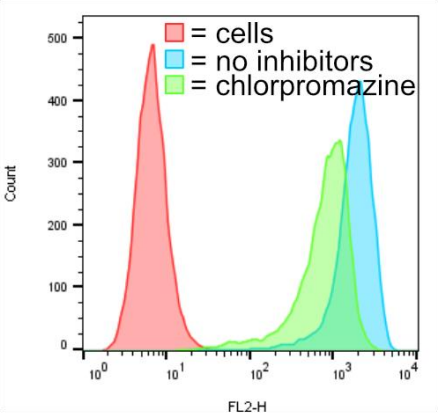
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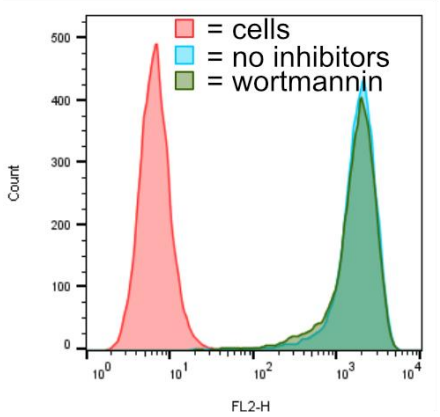
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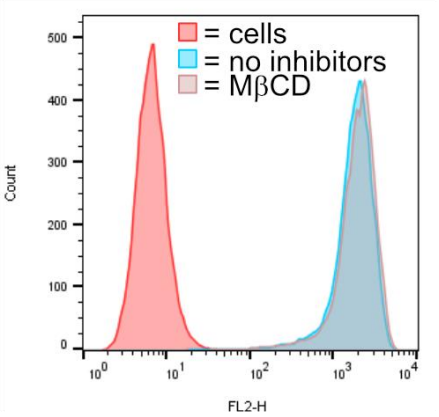
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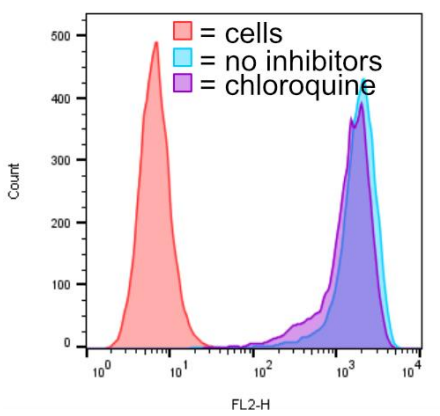
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Count
FL2-H

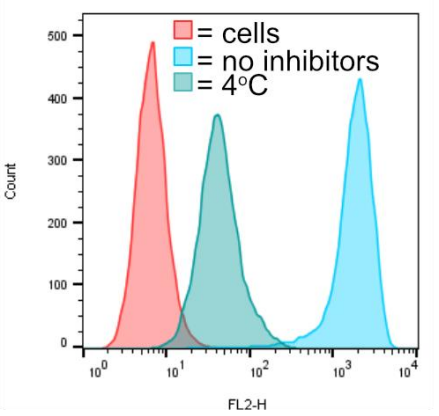

= cells
= no inhibitors
= 4°C
Count
FL2-H

**Figure S12**. P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) histograms for flow cytometry data in Figure 4C.

Perfluorocarbon nanoemulsions containing fluorous rhodamine (**S7**) were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) were added and emulsions were resonicated.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate. Cells were allowed to adhere (O/N). Media was replaced with basal DMEM and inhibitors: $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM) and incubated for 1 h (37 ˚C, 5% $CO_2$). Emulsions were added (10 μL) and incubated for a further 3 h (37 ˚C, 5% $CO_2$). For 4 ˚C, emulsions were added to cells and placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Error bars represent the standard deviation of 3 replicate experiments.

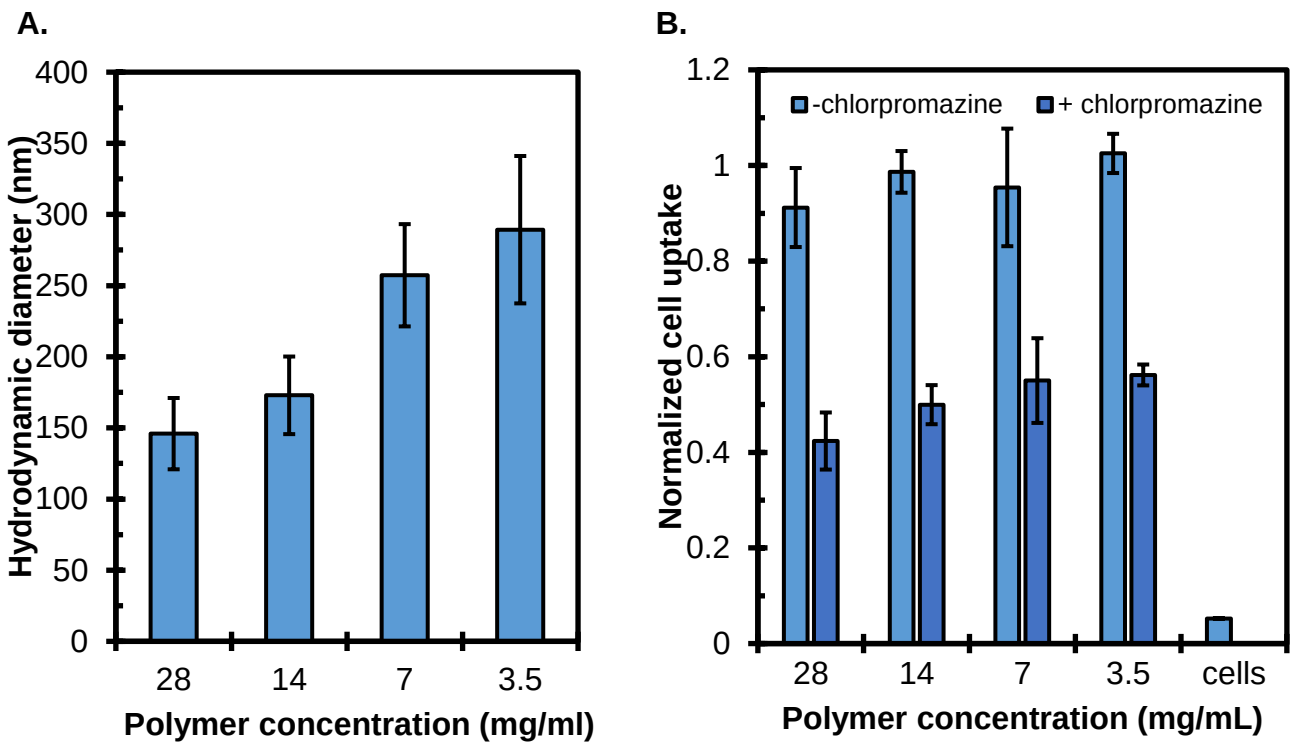
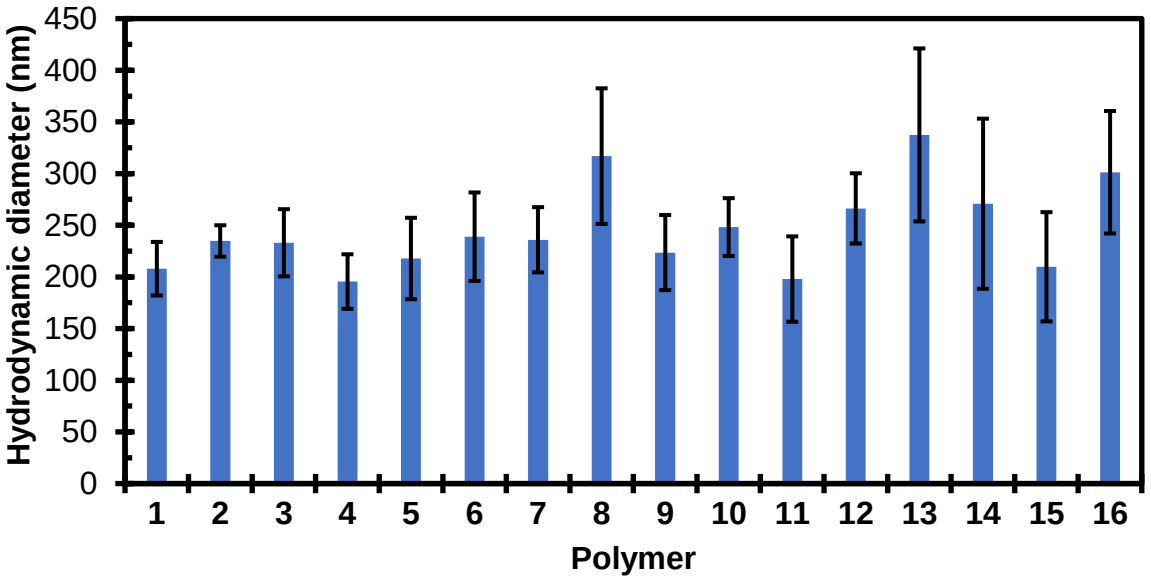



**Figure S13**. Cellular uptake of nanoemulsions ranging from 150–300 nm emulsions. (A) Hydrodynamic diameter of PFC nanoemulsions stabilized by different concentrations of P(MeOx$_{30}$-*b*-NonOx$_{10}$) (**5**). (B) RAW264.7 cell uptake of nanoemulsions in A in the presence (dark blue) or absence (light blue) of chlorpromazine.

(A) P(MeOx$_{30}$-*b*-NonOx$_{10}$) (**5**) emulsions were prepared following **the general emulsions formulation protocol** – varying the surfactant concentration from 3.5 mg/mL to 28 mg/mL**.** Prior to addition of fluorous rhodamine, the size was measured by following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples.

(B) RAW264.7 cells were plated in a 96-well plate (100,00 cells/well). Chlorpromazine (60 μM) was added to the cells and incubated for 1 h at 37 ˚C, 5% $CO_2$. After 1 h, emulsions (10 μL) were added to the cells and incubated for 3 h at 37 ˚C, 5% $CO_2$. Cells were then washed with media, LiCl buffer, PBS, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer with 15,000 cells collected per sample. Each respective polymer concentration was normalized to the "-chlorpromazine" treatment for that polymer concentration. Error bars represent the standard deviation of three replicate samples.

A.




B.

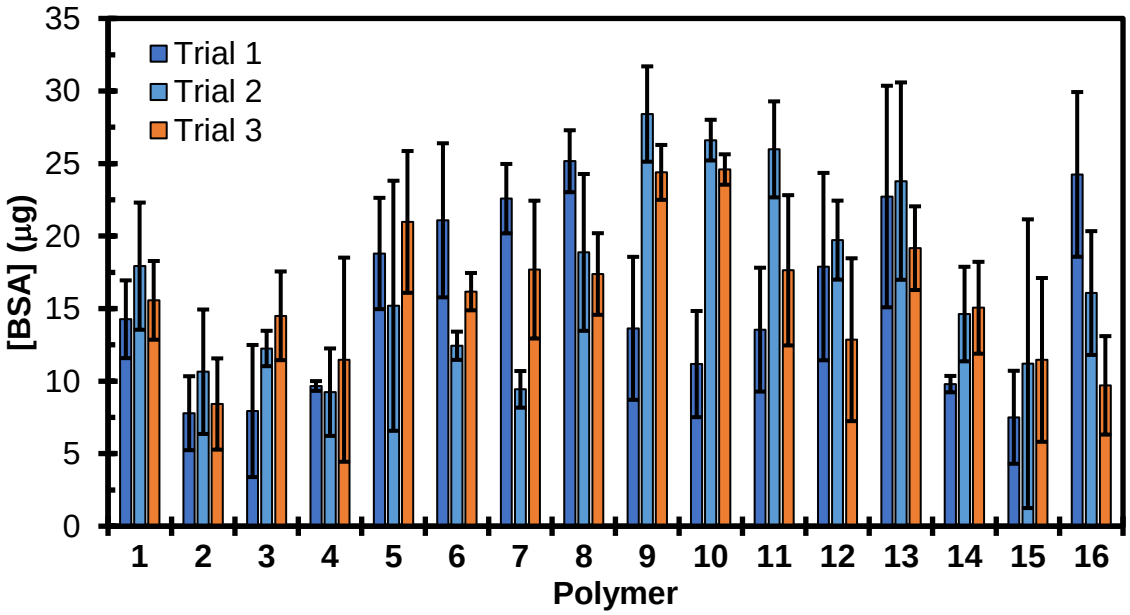



**Figure S14**. Adsorption of BSA on PFC nanoemulsions stabilized by polymer amphiphiles **1**—**16**. (A) Hydrodynamic diameter of PFC nanoemulsions. (B) Bradford analysis of protein adsorption on PFC nanoemulsions.

(A) Emulsions were prepared following **the general emulsions formulation protocol.** Size was measured following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples.

(B) Nanoemulsions were prepared following **the general emulsions formulation protocol** and subjected to 60 mg/mL BSA and rocked for 2 h at room temperature. Emulsions

were washed 2x via centrifugation (5.6 x *g*, 3 min). After washing, emulsions were destroyed by resuspending emulsions in dichloromethane (DCM, 100 μL) and probe sonicated for 15–30 s. Destroyed emulsions were dried, dissolved in deionized water (100 μL) and protein precipitated with cold acetone (2 x 400 μL). Following precipitation, protein is dissolved in deionized water (150 μL). Bradford assay was performed to quantify the protein with absorbance measurements at 595 nm. Each bar represents the average of three wells, for three independent trials (dark blue, light blue, and orange). Error bars represent the standard deviation of three wells for each independent trial.

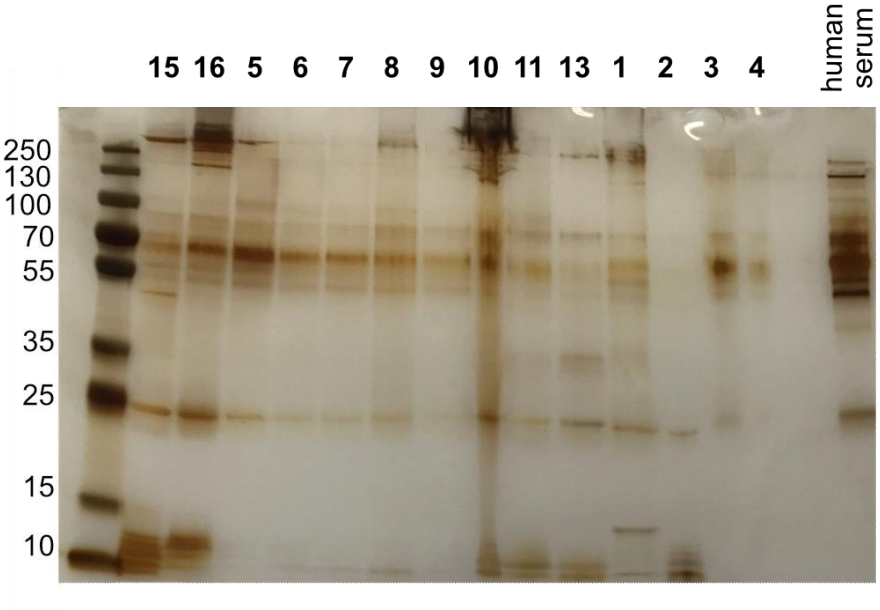



**Figure S15.** Silver stained SDS-PAGE gel representing protein adsorption of Human Serum on PFC nanoemulsions stabilized by polymer amphiphiles **1** (PF68), **2** (PF127), **3** (Zonyl FSO), **4** (Zonyl FSN), **5** [P(MeOx$_{30}$-*b*-NonOx$_{10}$)], **6** [P(MeOx$_{60}$-*b*-NonOx$_{10}$)], **7** [P(MeOx$_{90}$-*b*-NonOx$_{10}$)], **8** [P(MeOx$_{90}$-*b*-NonOx$_{10}$)], **9** [P(MeOx$_{30}$-*b*-NonOx$_{10}$-*b*-MeOx$_{30}$)], **10** [P(MeOx$_{90}$-*b*-NonOx$_{10}$-*b*-MeOx$_{90}$)], **11** [P(EtOx$_{30}$-*b*-NonOx$_{10}$)], **13** [P(EtOx$_{90}$-*b*-NonOx$_{10}$)], **15** (PEG$_{2K}$-*b*-NonOx$_{10}$), **16** (PEG$_{5K}$-*b*-NonOx$_{10}$).

Nanoemulsions (100 μL) prepared following **the general emulsions formulation protocol** were subjected to 10% Human Serum in PBS (1 mL) and rocked for 2 h at room temperature. The emulsions were washed 2x with PBS via centrifugation (5.6 x *g*, 3 min). After washing, emulsions were resuspended in Laemmli denaturing buffer and heated to 95 ˚C for 5 min. The emulsions were pelleted and the supernatant was loaded on a 12% SDS-PAGE gel. The gel was stained with silver stain.

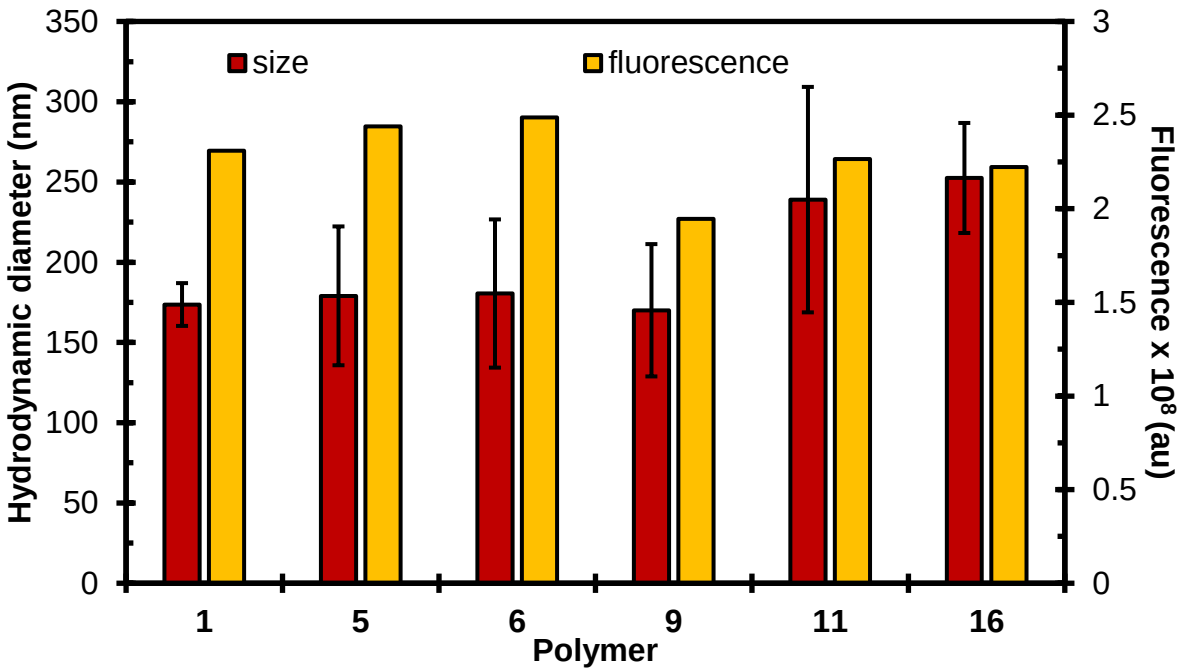



**Figure S16**. Cellular uptake of nanoemulsions stabilized by **1** (PF68), **5** [P(MeOx$_{30}$-*b*-NonOx$_{10}$)], **6** [P(MeOx$_{60}$-*b*-NonOx$_{10}$)], **9** [P(MeOx$_{30}$-*b*-NonOx$_{10}$-*b*-MeOx$_{30}$)], **11** [P(EtOx$_{30}$-*b*-NonOx$_{10}$)], **16** (PEG$_{5K}$-*b*-NonOx$_{10}$).

The emulsions above were prepared following **the general emulsions formulation protocol.** Size (red) was measured following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples. The fluorescence (yellow) of the emulsions were measured by diluting the emulsions 1:40 in a 1 cm quartz cuvette. Excitation: 500 nm, Emission collection: 525-650 nm, Integration: 0.1 sec, All slits: 2 nm

**B.**

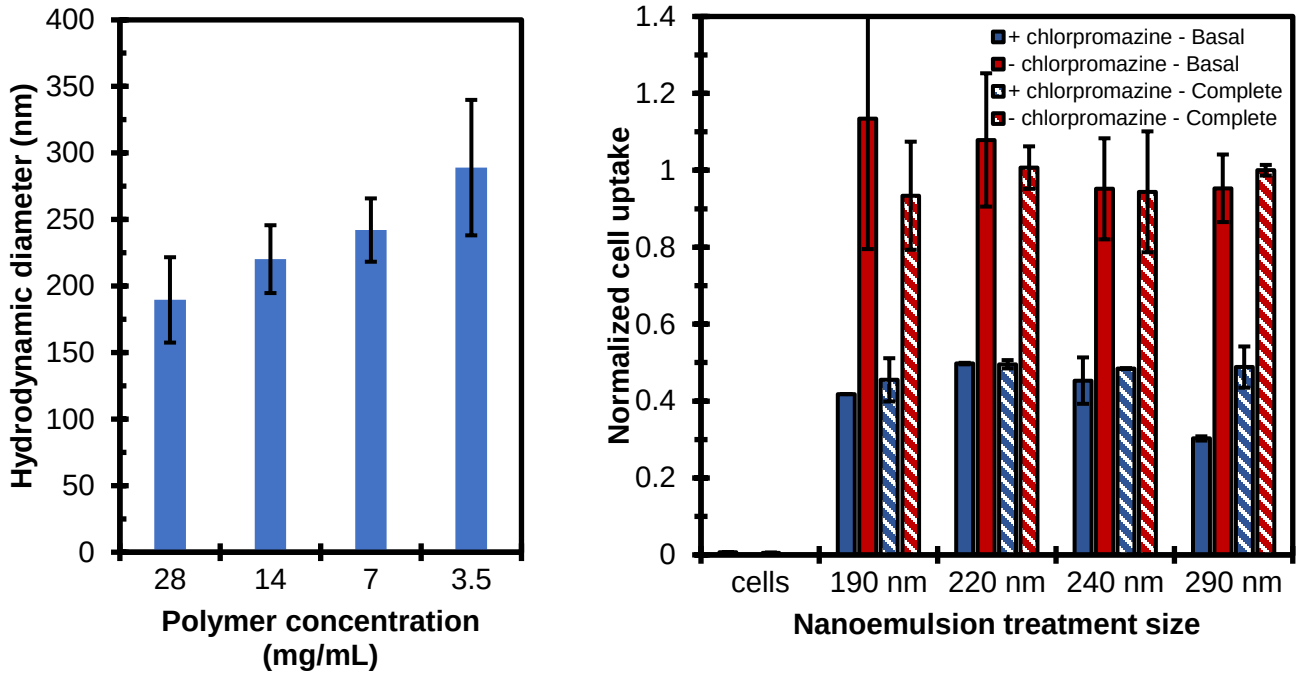



**Figure S17**. Cellular uptake of nanoemulsions ranging from 150—300 nm emulsions. (A) Hydrodynamic diameter of PFC nanoemulsions stabilized by different concentrations of P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**). (B) RAW264.7 cell uptake of nanoemulsions in A in the presence or absence of chlorpromazine and the presence (complete) or absence (basal) of protein.

(A) P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) emulsions were prepared following **the general emulsions formulation protocol.** Size was measured following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples.

(B) P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) emulsions were prepared following **the general emulsions formulation protocol** containing 3.7 μg of fluorous rhodamine. RAW264.7 cells were plated in a 96-well plate (100,000 cells/well). Media was removed and replaced with 100 μL of DMEM containing 10% FBS (+serum, + chlorpromazine, dashed blue), (+serum, -chlorpromazine, dashed red) or basal DMEM (-serum, + chlorpromazine, solid blue), (-serum, -chlorpromazine, solid red) and incubated at 37 ˚C, 5% $CO_2$ for 1 hour. Emulsions (10 μL) were added to the cells and incubated for 3 h at 37 ˚C, 5% $CO_2$. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Each emulsion size was normalized to one of the replicates in the samples without chlorpromazine. Error bars represent the standard deviation of 3 replicate samples.

## General experimental procedures

Chemical reagents were purchased from Sigma-Aldrich, Alfa Aesar, Fisher Scientific, or Acros Organics and used without purification unless noted otherwise. Anhydrous and deoxygenated dichloromethane (DCM), acetonitrile (MeCN), methanol (MeOH), and tetrahydrofuran (THF) were dispensed from a Grubb's-type Phoenix Solvent Drying System . Thin layer chromatography was performed using Silica Gel 60 F254 (EMD Millipore) plates. Flash chromatography was executed with technical grade silica gel with 60 Å pores and 40–63 μm mesh particle size (Sorbtech Technologies). Solvent was removed under reduced pressure with a Büchi Rotovapor with a Welch self-cleaning dry vacuum pump and further dried with a Welch DuoSeal pump. Bath sonication was performed using a Branson 3800 ultrasonic cleaner. Nuclear magnetic resonance ($^1$H NMR) spectra were taken on a Bruker Avance 500 instrument and processed with MestReNova software. All $^1$H NMR peaks are reported in reference to $CDCl_3$ at 7.26 ppm. Size Exclusion Chromatography (SEC), unless otherwise noted, was conducted on a Shimadzu high performance liquid chromatography (HPLC) system with a refractive index detector RID-10A, one Polymer Laboratories PLgel guard column, and two Polymer Laboratories PLgel 5 μm mixed D columns. Eluent was DMF with LiBr (0.1 M) at 50 °C (flow rate: 0.80 mL/ min). Calibration was performed using near-monodisperse poly(methyl-methacrylate) PMMA standards from Polymer Laboratories. Masses for analytical measurements were taken on a Sartorius MSE6.6S-000-DM Cubis Micro Balance. Microwave reactions were performed using a CEM Discover SP microwave synthesis reactor. All reactions were performed in glass 10 mL microwave reactor vials purchased from CEM with silicone/PTFE caps. Flea micro PTFE-coated stir bars were used in the vials with magnetic stirring set to high and 15 seconds of premixing prior to the temperature ramping. All microwave reactions were carried out at 140 °C with the pressure release limit set to 250 psi (no reactions exceeded this limit to trigger venting) and the maximum wattage set to 250W (the power applied was dynamically controlled by the microwave instrument and did not exceed this limit for any reactions).

## Abbreviations

BSA = Bovine Serum Albumin; βME = β-mercaptoethanol; DBC = diblock copolymer; DCM = dichloromethane; DLS = dynamic light scattering; DMEM = Dulbecco's modified eagle media; DMF = dimethyl formamide; DMSO = dimethyl sulfoxide; EDTA = Ethylenediaminetetraacetic acid; EtOH = ethanol; FACS = fluorescence activated cell sorting; FBS = fetal bovine serum; HLB = Hydrophilic lipophilic balance; HSA = Human Serum Albumin; LiCl = Lithium chloride; MβCD = methyl-β-cyclodextrin; MeCN = acetonitrile; MeOH = methanol; MeOTf = methyl triflate; $NaN_3$ = sodium azide; NMR = nuclear magnetic resonance; P(EtOx) = poly(2-ethyloxazoline); P(MeOx) = poly(2-methyloxazoline); P(NonOx) = poly(2-nonyloxazoline); PBS = Phosphate buffered saline; PDI = polydispersity index; PEG = poly(ethylene glycol) PenStrep = Penicillin streptomycin; PFC = perfluorocarbon; PFCE = perfluoro-15-crown-5-ether; PFD = perfluorodecalin; PFOB = perfluorooctyl bromide; PFTPA = perfluorotripropylamine; PMMA = poly(methyl-methacrylate); POx = poly(2-oxazoline); SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEC = size exclusion chromatography; TBC = triblock copolymer; THF = tetrahydrofuran; Tris-HCl = Tris(hydroxymethyl)aminomethane hydrochloride

**General polymer synthesis**

**Polymers 5-13**

To a flame dried microwave vial, MeCN (1.2 mL, anhydrous) and MeOx (**S1**) or EtOx (**S2**) were added. After brief mixing, MeOTf was added and the mixture was heated at 140 °C in the microwave. Note: block lengths were controlled via monomer to initiator ratio and reaction time.[7,8] Following completion of the first block, NonOx (**S3**) was added under $N_2$ and heated to 140 °C. After completion of the second block MeOx (**S1**) is added under $N_2$ and heated to 140 °C (**910**) or quenched with excess MilliQ water (**5**-**8**, **11**-**13**). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer as a white solid. Polymers are purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness

**Polymers 14-16**

To a flame dried microwave vial, MeCN and $PEG_n$-tosylate (**S4S6**) were added. After brief mixing, NonOx (**S3**) was added and the mixture was heated at 140 °C in the microwave. After polymerization was complete, the reaction was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer as a white solid. Polymers were purified by dissolving in DCM and washing against water, then further dialyzed against MeOH overnight, collected and evaporated to dryness.


(7) Lava, K.; Verbraeken, B.; Hoogenboom, R. Poly ( 2-Oxazoline ) s and Click Chemistry : A Versatile Toolbox toward Multi-Functional Polymers. *Eur. Polym. J.* **2015**, *65*, 98–111.

(8) Hoogenboom, R.; Wiesbrock, F.; Huang, H.; Leenen, M. A. M.; Thijs, H. M. L.; Van Nispen, S. F. G. M.; Van Der Loop, M.; Fustin, C. A.; Jonas, A. M.; Gohy, J. F.; Schubert, U. S. Microwave-Assisted Cationic Ring-Opening Polymerization of 2-Oxazolines: A Powerful Method for the Synthesis of Amphiphilic Triblock Copolymers. *Macromolecules* **2006**, *39*, 4719–4725.


**General nanoemulsion formation procedure**

Polymer surfactant (5.6 mg, 2.8 wt%) was dissolved in cosolvent (20 µL, DMF, MeOH or THF) and sonicated in a bath sonicator (~15 minutes) until fully dissolved, at which point 7:3 perfluorodecalin : perfluorotripropylamine (10 vol%, 20 µL) was added, followed by PBS buffer pH 7.4 (200 µL). Pluronic F-68 (**1**), Pluronic F-127 (**2**), Zonyl FSO (**3**), Zonyl FSN (**4**) required no cosolvent. $P(MeOx_x\text{-}b\text{-}NonOx_y\text{-}b\text{-}MeOx_z)$ (**5**–**10**), $P(EtOx_x\text{-}b\text{-}NonOx_y)$ (**11**–**13**) and $PEG_{1K}\text{-}b\text{-}NonOx_{10}$ (**14**) were dissolved in DMF. $PEG_{2K}\text{-}b\text{-}NonOx_{10}$ (**15**) and $PEG_{5K}\text{-}b\text{-}NonOx_{10}$ (**16**) were dissolved in THF and MeOH respectively. The mixture was sonicated at 35% amplitude for 90 seconds at 0 °C on a QSonica (Q125) sonicator. For $P(EtOx_x\text{-}b\text{-}NonOx_y)$ (**11**–**13**) and $PEG_n\text{-}b\text{-}NonOx_m$ (**14**–**16**) polymers the mixture was sonicated at 35% amplitude for 90 seconds pulsed on for 2 seconds, off for 10 seconds at 0°C. Sonication was performed by lowering the probe directly at the liquid-liquid interface of the two immiscible solvents. To remove cosolvents, emulsions are washed by centrifugation (5.6 x *g*, 3 min, 2x).

**Nanoemulsion size analysis**

The bulk emulsion solution was diluted in MilliQ $H_2O$ (20 µL emulsions in 2 mL MilliQ $H_2O$) in a plastic 1 cm cuvette. Size was analyzed with a Malvern Zetasizer Nano dynamic light scattering. Standard operating procedure parameters: 10 runs, 10 seconds/run, three measurements, no delay between measurements, 25 °C with 120 second equilibration time. Collection parameters:

Lower limit = 0.6, Upper limit = 1000, Resolution = High, Number of size classes = 70, Lower size limit = 0.4, Upper size limit = 1000, Lower threshold = 0.05, Upper threshold = 0.01. Data is representative of three replicate measurements. Size error bars represent the product of the dispersity and the z-average of the measurements.

**Payload release experiment**

Perfluorocarbon nanoemulsions (**1**-**16**) containing fluorous coumarin **17** were prepared by dissolving coumarin in acetone to make a stock solution (2.3 mg/mL). Coumarin **17** (0.05 mg, 0.04 μmol, 20 μL) was then partioned to eppendorf tubes and the acetone was dried. Once dried, perfluorocarbons (7:3 PFD : PFTPA, 20 μL) were added to dissolve the coumarin, and deionized water (200 μL) was added. Separately, the polymers were dissolved with required cosolvent. The PFC/water mixture was placed on the sonication probe, and immediately before starting the probe, the polymer solution (see general nanoemulsion formation procedure) was added. The mixture was sonicated for 90s either continuously or pulsed as described in the general nanoemulsion formation procedure. Immediately after formation, emulsions solution (40 μL) was diluted with PBS (960 μL). 1-octanol (500 μL ) was layered on top of the water and placed on an orbital rocker at 40 rpm.

The 1-octanol (250 μL) was removed with a syringe (250 μL Hamilton) at 3 h, 1 day, 3 days, 7 days, 10 days, and 14 days and the fluorescence was measured in a 0.3 cm cuvette as described in the general photophysics procedures. After measurement, the 1-octanol was then carefully replaced to minimize loss during transfer and placed back on the rocker until next measurement.

The control was fluorous coumarin **17** (3.2 μL, 0.007 mg, 6.1 nmol) dissolved in 1-octanol (500 μL) directly, and bath sonicated for 10 min to dissolve. This is the amount of fluorous coumarin that is expected to come into contact with the 1-octanol after the emulsions were diluted with PBS.

Fluorimeter settings : Path length: 0.3 cm, Exc: 375 nm, collect: 400-700 nm, all slits: 2nm, Integration: 0.1 sec.

**General photophysics procedures**

Photoluminescence spectra were obtained on a Horiba Instruments PTI QuantaMaster Series fluorometer. Quartz cuvettes (0.3 cm path length) were used for photoluminescence measurements unless otherwise noted.

**General cell culture procedures**

RAW264.7 cells were purchased from ATCC (Cat# TIB-71).

RAW264.7 cells were cultured in Dulbecco's Modified Eagle Media (DMEM, Life Technologies, cat# 11995073) supplemented with 10% fetal bovine serum (Corning, lot# 35016109) and 1% penicillin-streptomycin (Life Technologies, cat# 15070063). Cells were washed with PBS, or PBS supplemented with 1% fetal bovine serum (FBS, FACS buffer). Cells were incubated at 37 ˚C, 5% $CO_2$, during treatments and throughout culturing, in HERACell 150i CO2 incubators. Cells were pelleted through use of Sorvall ST 40R centrifuge. All cell work was performed in 1300 Series A2 biosafety cabinets.

For cell viability experiments: following incubation, cells were washed three times by centrifugation (526 x *g*, 3 min, 4˚C). Propidium iodide solution (2 □L in 1 mg/mL in PBS) was

added to each well. Cells/PI were transferred to FACS tubes with a final volume of 200 μL FACS buffer (PBS + 1% FBS). Cells were incubated on ice for 15 minutes prior to flow cytometry measurement. PI fluorescence was measured on FL2 channel. Data were analyzed by splitting the population at $10^2$ as a live/dead line. Flow cytometry was performed on a BDBiosciences FACSCalibur equipped with 488 nm and 635 nm lasers. For assessment of the statistical significance of differences, a one-tailed Student's t-test assuming unequal sample variance was employed.

For inhibition experiments: Media was removed and replaced with 100 μL of DMEM (either basal or complete) and inhibitors $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM). After 1 h, emulsions (10 μL) were added to the cells and incubated for 3 h at 37 ˚C, 5% $CO_2$. For 4 ˚C, emulsions were added to cells and placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, sterile Lithium Chloride buffer (LiCl: 0.25 M LiCl, 1 mM EDTA, 10 mM Tris-HCl) 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate. Note: PFC nanoemulsions are dense and settle on top of cells. Slight rocking was necessary to successfully remove excess emulsions. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Each emulsion was normalized to one of the replicates in the samples with no inhibitors present. Error bars represent the standard deviation of 3 replicate experiments.

Confocal microscopy was performed on a TCS SPE Leica confocal microscope containing 405 nm, 488 nm, 532 nm and 635 nm lasers.

**Figure experimental procedures**

**Figure 2C/D**.
Perfluorocarbon nanoemulsions (**1**, **5–6**, **9**, **16**) were prepared following the **general nanoemulsion formation procedure** containing either perfluorooctyl bromide (PFOB), perfluoro-15-crown-5-ether (PFCE) or, 7:3 perfluorodecalin (PFD) perfluorotripropylamine (PFTPA) (10 vol%, 20 μL). Size was measured on day 0, day 1, day 2, day 3, day 7, and day 14 by resuspending emulsions via vortexing briefly (~5 s) and pipetting up and down, then diluting 1:1000 following **nanoemulsion size analysis**. Reported is the change in volume from day 0 to day 14. Error bars represent the standard deviation of the measurements.
$V = \frac{4}{3}\pi r^3$ where r = radius of nanoemulsions

**Figure 2E/F.**
Perfluorocarbon nanoemulsions stabilized by each surfactant (**1**–**16**) were prepared as described in the **general nanoemulsion formation procedure**. Emulsion size was then monitored on day 1, 2, 3, 7, 14, and 30 following the **general nanoemulsions analysis procedure**. Bars represent the average of three samples and error bars represent the standard deviation of three samples.

Volume is a more accurate description of Ostwald ripening over time. Thus, diameter on day 0 and day 30 were converted to volume of a sphere
$V = \frac{4}{3}\pi r^3$
where r is radius. Bars represent the difference of volume on day 30 and day 0 of three samples. Error bars represent the propagation of error of three separate samples.

**Figure 3B.**
Perfluorocarbon nanoemulsions (**1–16**) containing fluorous coumarin **17** were prepared by dissolving coumarin in acetone to make a stock solution (2.3 mg/mL). Coumarin **17 (**0.05 mg, 0.04 μmol, 20 μL) was then partioned to eppendorf tubes and the acetone was dried. Once dried, perfluorocarbons (7:3 PFD : PFTPA, 20 μL) were added to dissolve the coumarin, and deionized water (200 μL) was added. Separately, the polymers were dissolved with required cosolvent. The PFC/water mixture was placed on the sonication probe, and immediately before starting the probe, the polymer solution (see **general nanoemulsion formation procedure**) was added. The mixture was sonicated for 90s either continuously or pulsed as described in the **general nanoemulsion formation procedure**. Immediately after formation, emulsions solution (40 μL) was diluted with PBS (960 μL). 1-octanol (500 μL) was layered on top of the water and placed on an orbital rocker at 40 rpm.

The 1-octanol (200 μL) was removed with a syringe (250 μL Hamilton) at 3 h, 1 day, 3 days, 7 days, 10 days, and 14 days and the fluorescence was measured in a 0.3 cm cuvette as described in the **general photophysics procedures**. After measurement, the 1-octanol was then carefully replaced to minimize loss during transfer and placed back on the rocker until next measurement.

The control was fluorous coumarin **17** (3.2 μL, 0.007 mg, 6.1 nmol) dissolved in 1-octanol (500 μL) directly, and bath sonicated for 10 min to dissolve. This is the amount of fluorous coumarin

that is expected to come into contact with the 1-octanol after the emulsions were diluted with PBS.

Fluorimeter settings : Path length: 0.3 cm, Exc: 375 nm, collect: 400-700 nm, all slits: 2nm, Integration: 0.1 sec.

**Figure 4A / Figure S4.**
Perfluorocarbon nanoemulsions (**6**, **10**, **16**) containing fluorous rhodamine (**S7**) were prepared by preparing nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) were added and emulsions are resonicated.

RAW264.7 cells (30,000 cells/well) were plated on a μ-Slide 8 Well ibiTreat tissue culture treated slides (Ibidi Cat# 80826). Cells were allowed to adhere overnight. Media was replaced with complete DMEM and treated with emulsions (40 μL, P($MeOx_{60}$-*b*-$NonOx_{10}$) (**6**), P($EtOx_{30}$-*b*-$NonOx_{10}$) (**11**), or $PEG_{5K}$-*b*-$NonOx_{10}$ (**16**)). Treatment was incubated for 3 h (37 ˚C, 5% $CO_2$). After treatment, cells were washed with media 3x, LiCl buffer 3x and replaced with OptiMEM containing cellular stains. PFC emulsions are dense and settle onto the cells, to remove the emulsions, slight rocking was necessary. Cells were stained with Hoechst (3.24 □M, ThermoFisher Cat# PI62249), LysoTracker Green DND-26 (200 nM, Cell Signaling Technologies Cat# 8783S) in OptiMEM. Cells were incubated with Hoescht at room temperature for 30 minutes prior to imaging, Hoescht in OptiMEM was removed and replaced with OptiMEM containing LysoTracker stains and imaged immediately.

Confocal microscopy was performed on a TCS SPE Leica confocal microscope containing 405 nm, 488 nm, 532 nm and 635 nm lasers. Confocal settings were as follows: Hoechst (405 laser-50%, 800 gain, offset -0.4, collection 420-500nm), LysoTracker Green DMD-26 (488 laser-50%, 800 gain, offset -0.4, collection 500-550 nm), Rhodamine (532 laser-50%, 800 gain, offset -0.4, collection 540-700nm), DIC (scan-BF, 450 gain, offset -0.4). Scale bar represents 7.5 μm. Images were processed in ImageJ.

**Figure 4C-F.**
Perfluorocarbon nanoemulsions (**1**–**16**) containing fluorous rhodamine **S7** were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine **S7** dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were resonicated.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate (Fisher, Cat# 07-201-94). Cells were allowed to adhere overnight. Media was replaced with basal DMEM and inhibitors: $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM) and incubated for 1 h (37 ˚C, 5% $CO_2$). Emulsions were added (10 μL) and incubated for a further 3 h (37 ˚C, 5% $CO_2$). For 4 ˚C, emulsions were added to cells and placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate (Fisher, Cat# 07-200-96). Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed

by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Error bars represent the standard deviation of three replicate experiments.

**Figure 5B/Figure S14.**
Perfluorocarbon nanoemulsions (**1**–**16**) were prepared by following the **general nanoemulsion formulation procedure**. After washing, emulsions (100 μL) were diluted with deionized water (1 mL DI $H_2O$) or 60 mg/mL BSA dissolved in water (1 mL). Emulsions were rocked on an orbital rocker for 2 h at 40 rpm. Following treatment, emulsions are washed with DI $H_2O$ by centrifugation 2x (5.6 x *g*, 3 min), to remove unbound protein. Following the last wash, emulsions were resuspended in dichloromethane (DCM, 100 μL) and sonicated with the probe sonicator for 30 s. The DCM was then evaporated to leave a film of polymer and protein. Protein was then dissolved in DI $H_2O$ (100 μL) and cold acetone (400 μL). The mixture was cooled for 2 h (-20 ˚C) to precipitate the protein. The tubes were then centrifuged (17.7 x *g*, 10 min), to pellet protein. The supernatant was removed and the process was repeated to precipitate the protein again. Following precipitation, samples were dissolved in DI $H_2O$ (150 μL).

Protein concentration was measured via Bradford assay. A standard curve of BSA from 0–30 μg/mL was made following manufacturer specifications (VWR, Cat# P123200). Samples were measured by diluting 10 μL sample with 290 μL Bradford reagent. Absorbance was measured on plate reader at 595 nm.

Absorbance of emulsions treated with DI $H_2O$ was subtracted from absorbance of emulsions treated with BSA. Data represents the average of three independent experiments, each experiment was performed in triplicate. Error bars represent the standard deviation of the trials.

**Figure 5C.**
Perfluorocarbon nanoemulsions **(1–12**, **14**–**15**) were prepared by following the **general nanoemulsion formulation procedure**. After washing, emulsions (100 μL) were diluted with complete DMEM (1 mL, 10 % FBS, 1% PenStrep). Emulsions were rocked on an orbital rocker for 2 h at 40 rpm. Following treatment, emulsions are washed with DI $H_2O$ by centrifugation 2x (5.6 x *g*, 3min), to remove unbound protein. Following the last wash, emulsions were resuspended in Laemmli buffer (BioRad, Cat# 1610737) containing β-mercaptoethanol (βME) and heated (95 ˚C, 5 min) to denature proteins. Emulsions were pelleted (5.6 x *g*, 3 min), and supernatant was loaded onto 12% SDS-PAGE gel (BioRad, Cat# 5671043). The gel was run at 120 V for 50 min, and stained with silver stain (ThermoFisher, Cat# PI24612) for visualization.

**Figure 6A/Figure S16.**
Perfluorocarbon nanoemulsions (**1**, **5–6**, **9**, **11**, **16**) containing fluorous rhodamine were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) is added and emulsions are re-sonicated. Emulsions (100 μL) were treated with either 60 mg/mL BSA in $H_2O$ (+ serum) or $H_2O$ (- serum) (1 mL each). The emulsions were rocked for 2 hours at room temperature, washed by centrifugation (3.0 rpm, 3 min, 3x), resuspended in 100 μL of $H_2O$ and added to cells.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate (Fisher, Cat# 07-201-94). Cells were allowed to adhere (O/N). Media was replaced with 100 μL of basal media.

Emulsions (+/- serum) were added and incubated for 3 h (10 μL, 37 ˚C, 5% $CO_2$). Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate (Fisher, Cat# 07-200-96). Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Error bars represent the standard deviation of three replicate experiments.

**Figure 6B/Figure S17.**
Perfluorocarbon nanoemulsions (**1**, **5–6**, **9**, **11**, **16**) containing fluorous rhodamine **24** were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine **24** dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were re-sonicated.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate (Fisher, Cat# 07-201-94). Cells were allowed to adhere (O/N). Media was replaced with complete DMEM and inhibitors: $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM) and incubated for 1 h (37 ˚C, 5% $CO_2$). Emulsions were added (10 μL) and incubated for a further 3 h (37 ˚C, 5% $CO_2$). For 4 ˚C, emulsions were added to cells and placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate (Fisher, Cat# 07-200-96). Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Error bars represent the standard deviation of three replicate experiments.

**Supplemental Figure Experimental**

**Figure S1.**
Perfluorocarbon nanoemulsions (**1**–**5**, **7**–**9**, **11**–**13**) were prepared through ultrasonication (35% amp, 90 sec) of solutions containing 7:3 perfluorodecalin (PFD) perfluorotripropylamine (PFTPA) (10 vol%, 20 μL) and polymer surfactant 28 mg/mL (2.8 wt%, blue), 14 mg/mL (1.4 wt%, red), 7 mg/mL (0.7 wt%, orange), and 3.5 mg/mL (0.35 wt%, grey) in phosphate buffered saline (PBS, pH 7.4, 200 μL). Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples. Size was measured following the **nanoemulsion size analysis**.

Stock solutions of PF68 (**1**), PF127 (**2**), Zonyl FSN-100 (**3**), and Zonyl FSO (**4**) were made in PBS (phosphate buffered saline) pH 7.4 at 28 mg/mL. Stock solutions of the POx polymers (**7**–**9**, **11**–**13**) were made in PBS with 10% DMF at 28 mg/mL.

**Figure S2.**
Perfluorocarbon nanoemulsions (**1–16**) were prepared through ultrasonication following the **general nanoemulsion formation procedure**. Size was measured on day 0 (grey), day 1 (purple), day 2 (blue), day 3 (green), day 7 (yellow), day 14 (orange), and day 30 (red) by resuspending emulsions via vortexing briefly (~5 s) and pipetting up and down, following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples.

Stock solutions of PF68 (**1**), PF127 (**2**), Zonyl FSN-100 (**3**), and Zonyl FSO (**4**) were made in PBS (phosphate buffered saline) pH 7.4 at 28 mg/mL. Stock solutions of the POx polymers were made in PBS with 10% DMF at 28 mg/mL. Stock solutions of $PEG_{1K}$-*b*-NonOx (**14**), $PEG_{2K}$-*b*-NonOx (**15**), and $PEG_{5K}$-*b*-NonOx (**16**) were made in PBS with 10% MeOH, THF, and DMF respectively at 28 mg/mL.

**Figure S5.**
Perfluorocarbon nanoemulsions (**1**, **5–6**, **11**, **16**) containing fluorous rhodamine **24** were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine **24** dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were resonicated.

RAW264.7 cells (30,000 cells/well) were plated on a μ-Slide 8 Well ibiTreat tissue culture treated slides (Ibidi Cat# 80826). Cells were allowed to adhere overnight. Media was replaced with complete DMEM and treated with emulsions (**1**, **5–6**, **11**, **16**). Treatment was incubated for 3 h (37 ˚C, 5% $CO_2$). After treatment, cells were washed with media 3x, LiCl buffer 3x and replaced with OptiMEM containing cellular stains. PFC emulsions are dense and settle onto the cells, to remove the emulsions, slight rocking was necessary. Cells were stained with Hoechst (3.24 μM, ThermoFisher Cat# PI62249), LysoTracker Green DND-26 (200 nM, Cell Signaling Technologies Cat# 8783S). Cells were incubated with Hoescht at room temperature for 30 minutes prior to imaging, Hoescht in OptiMEM was removed and replaced with OptiMEM containing LysoTracker stains and imaged immediately.

PFC nanoemulsions stabilized by polymer surfactants (**1**, **5–6**, **11**, **16**) were labeled with fluorous rhodamine (red – B, E, H, K, N). Cells were stained with Hoescht (blue) and LysoTracker (green – C, F, I, L, O). Cells were imaged via excitation at 488 nm to visualize the LysoTracker green (C), 532 nm to visualize the rhodamine (D), and merged to show colocalization (A, D, G, J, M). Scale bars represent 7.5 μm.

Confocal settings were as follows: Hoechst (405 laser-50%, 800 gain, offset -0.4, collection 420-500nm), LysoTracker Green DMD-26 (488 laser-50%, 800 gain, offset -0.4, collection 500-550 nm), Rhodamine (532 laser-50%, 800 gain, offset -0.4, collection 540-700nm), DIC (scan-BF, 450 gain, offset -0.4). Scale bar represents 7.5 μm. Images were processed in ImageJ. Confocal microscopy was performed on a TCS SPE Leica confocal microscope containing 405 nm, 488 nm, 532 nm and 635 nm lasers.

**Figure S7.**
Media was removed and replaced with 100 μL of basal DMEM containing inhibitors $NaN_3$ (10 mM), Chlorpromazine (60 μM), Wortmannin (0.4 μM), MβCD (20 μM), Chloroquine (100 μM). Cells were incubated with inhibitors for 4 h at 37 ˚C, 5% $CO_2$. Cells were placed at 4 ˚C for 1 h to prevent excessive cell death. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate. Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Cells were stained with propidium iodide (2 μL, 1 mg/mL, 15 min) on ice. Viability was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Error bars represent the standard deviation of three replicate experiments.

**Figure S8.**
Perfluorocarbon nanoemulsions stabilized by (P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) and $PEG_{5K}$-*b*-$NonOx_{10}$ (**16**)) containing fluorous rhodamine **24** were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were resonicated.

RAW264.7 cells (30,000 cells/well) were plated on a μ-Slide 8 Well ibiTreat tissue culture treated slides (Ibidi Cat# 80826). Cells were allowed to adhere (O/N). Media was replaced with either basal DMEM or basal DMEM and chlorpromazine (60 μM). Cells were incubated with chlorpromazine for 1 h before addition of emulsions and then further incubated for 3 h at 37 ˚C, 5% $CO_2$. After treatment, cells were washed with media 3x, LiCl buffer 3x and replaced with OptiMEM containing cellular stains. PFC emulsions are dense and settle onto the cells, to remove the emulsions, slight rocking was necessary. Cells were stained with Hoechst (3.24 μM, ThermoFisher Cat# PI62249), LysoTracker Green DND-26 (200 nM, Cell Signaling Technologies Cat# 8783S). Cells were incubated with Hoescht at room temperature for 30 minutes prior to imaging, Hoescht in OptiMEM was removed and replaced with OptiMEM containing LysoTracker stains and imaged immediately. Scale bar represents 25 μm.

ImageJ was used to analyze the total pixel intensity of the emulsions (rhodamine channel, red). Bars represent the average of three independent images, and error bars represent the standard deviation.

**Figure S10**.
Perfluorocarbon nanoemulsions stabilized by **1**–**16** containing fluorous rhodamine were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine dissolved in acetone (0.4 □L of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were resonicated.

RAW264.7 cells (100,000 cells/well) were plated on a flat bottom 96-well plate (Fisher, Cat# 07-201-94). Cells were allowed to adhere (O/N). Media was replaced with complete DMEM and inhibitors: Wortmannin (0.4 μM, purple), MβCD (20 μM, black). Cells were incubated with inhibitors for 1 h at 37 ˚C, 5% $CO_2$. After 1 h, emulsions (10 μL) were added to the cells and incubated for 3 h at 37 ˚C, 5% $CO_2$. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate (Fisher, Cat# 07-200-96). Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Each emulsion was normalized to no inhibitors present. Error bars represent the standard deviation of 3 replicate experiments.

**Figure S13**.
P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) emulsions containing fluorous rhodamine were prepared by making nanoemulsions following the **general nanoemulsion formulation procedure**. After washing by centrifugation, fluorous rhodamine **24** dissolved in acetone (0.4 μL of 9.2 mg/mL stock, 0.4 mg) was added and emulsions were resonicated.

A. P($MeOx_{30}$-*b*-$NonOx_{10}$) (**5**) emulsions were prepared following **the general nanoemulsion formulation protocol.** Prior to addition of fluorous rhodamine, size was measured by following the **nanoemulsion size analysis**. Error bars represent the average of the product of the $Z_{average}$ and the polydispersity index of three replicate samples.
B. RAW264.7 cells were plated in a 96-well plate (100,00 cells/well). Chlorpromazine (60 μM) was added to the cells and incubated for 1 h at 37˚C, 5% $CO_2$. After 1 h, emulsions (10 μL) were added to the cells and incubated for 3 h at 37˚C, 5% $CO_2$. Cells were then washed with media 3x, LiCl buffer 3x, PBS 1x, then lifted with trypsin and transferred to a v-bottom 96-well plate (Fisher, Cat# 07-200-96). Cells were washed by centrifugation (2x, 526 x *g*, 3 min) and resuspended in FACS buffer (PBS + 1% FBS) to a final volume of 200 μL. Uptake was analyzed by FL2 channel on a FACSCalibur flow cytometer. 15,000 cells were collected per sample. Each emulsion size was normalized to one of the replicates in the samples without chlorpromazine. Error bars represent the standard deviation of 3 replicate samples.

**Figure S15**.
Perfluorocarbon nanoemulsions (**1**–**13**, **15**–**16**) were prepared by following the **general nanoemulsion formulation procedure**. After washing, emulsions (100 μL) were diluted with 10% human serum diluted in PBS (1 mL, pH 7.4). Emulsions were rocked on an orbital rocker for 2 h at 40 rpm. Following treatment, emulsions were washed with DI $H_2O$ by centrifugation 2x (3 rpm, 3 min), to remove unbound protein. Following the last wash, emulsions were resuspended in Laemmli buffer (BioRad, Cat# 1610737) containing β-mercaptoethanol (βME) and heated (95 ˚C, 5 min) to denature proteins. Emulsions were pelleted (5.6 x *g*, 3 min), and

the supernatant was loaded onto 12% SDS-PAGE gel (BioRad, Cat# 5671043). The gel was stained with silver stain (ThermoFisher, Cat# PI24612) for visualization.

**Polymer synthesis and characterization**

P(MeOx$_{32}$-*b*-NonOx$_{9}$) (**5**) (Figure S18, S30)
To a flame dried microwave vial, MeCN (1.2 mL, anhydrous) and MeOx (200 μL, 0.200 g, 2.40 mmol, 30 equiv.) were added. After brief mixing, MeOTf (8.9 μL, 13 mg, 0.078 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 7 minutes, NonOx (155 μL, 155 mg, 0.783 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C for 3 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**5**) as a white solid. Polymer **5** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (102 mg, 0.022 mmol, 29% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 162H), 3.02 (m, 3H), 2.32 (m, 25H), 2.13 (m, 98H), 1.56 (m, 18H), 1.24 (m, 106H) 0.85 (t, *J* = 6.6 Hz, 26H). SEC: $M_w$ = 5.0 kDa, $M_n$ = 4.1 kDa, $Đ$ = 1.24.

P(MeOx$_{62}$-*b*-NonOx$_{8}$) (**6**) (Figure S19, S30)
To a flame dried microwave vial, MeCN (2.1 mL, anhydrous) and MeOx (360 μL, 0.360 g, 4.32 mmol, 60 equiv.) were added. After brief mixing, MeOTf (8.0 μL, 12 mg, 0.072 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 16 minutes, NonOx (139 μL, 139 mg, 0.702 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C for 15 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**6**) as a white solid. Polymer **6** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (400 mg, 0.057 mmol, 80% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 284H), 3.03 (m, 3H), 2.13 (m, 18H), 1.97 (m, 187H), 1.58 (m, 17H), 1.25 (m, 99H) 0.86 (t, *J* = 6.6 Hz, 25H). SEC: $M_w$ = 8.5 kDa, $M_n$ = 7.4 kDa, $Đ$ = 1.16.

P(MeOx$_{87}$-*b*-NonOx$_{7}$) (**7**) (Figure S20, S30)
To a flame dried microwave vial, MeCN (2.4 mL, anhydrous) and MeOx (400 μL, 0.400 g, 4.80 mmol, 90 equiv.) were added. After brief mixing, MeOTf (5.9 μL, 9 mg, 0.053 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 24 minutes, NonOx (103 μL, 103 mg, 0.519 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C for 21 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**7**) as a white solid. Polymer **7** was purified by precipitation by dissolving in a minimal amount of DCM and dropwise addition to cold $Et_2O$ (20:1 v/v%), collected and evaporated to dryness (462 mg, 0.053 mmol, 92% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.43 (m, 376H), 3.03 (m, 3H), 2.13 (m, 307H), 1.58 (m, 15H), 1.24 (m, 70H) 0.86 (t, *J* = 6.6 Hz, 20H). SEC: $M_w$ = 12.5 kDa, $M_n$ = 11.1 kDa, $Đ$ = 1.13.

P(MeOx$_{96}$-*b*-NonOx$_{26}$) (**8**) (Figure S21, S30)
To a flame dried microwave vial, MeCN (0.75 mL, anhydrous) and MeOx (250 μL, 0.25 g, 3.00 mmol, 90 equiv.) were added. After brief mixing, MeOTf (3.7 μL, 5.6 mg, 0.033 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 14 minutes, NonOx (193 μL, 193 mg, 0.972 mmol, 30 equiv.) was added under $N_2$ and heated to 140 °C for 15 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**8**) as a white solid. Polymer **8** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and

evaporated to dryness (422 mg, 0.032 mmol, 95% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.45 (m, 488H), 3.04 (m, 3H), 2.12 (m, 360H), 1.57 (m, 51H), 1.24 (m, 287H) 0.85 (t, *J* = 6.6 Hz, 77H). SEC: $M_w$ = 11.4 kDa, $M_n$ = 10.1 kDa, *Đ* = 1.14.

P(MeOx$_{31}$-*b*-Non$_{10}$-*b*-MeOx$_{31}$) (**9**) (Figure S22, S31)
To a flame dried microwave vial, MeCN (0.88 mL, anhydrous) and MeOx (150 μL, 0.150 g, 1.8 mmol, 30.0 equiv.) were added. After brief mixing, MeOTf (6.7 μL, 9.7 mg, 0.060 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 8 minutes, NonOx (116 μL, 116 mg, 0.586 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C. After 8 minutes, MeOx (150 μL, 0.15 g, 1.8 mmol, 30.0 equiv.) was added under $N_2$ and heated to 140 °C for 9 minutes, at which point the polymerization was quenched with MilliQ water (excess). The reaction mixture was evaporated to dryness to yield crude polymer (**9**) as a white solid. Polymer **9** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (355 mg, 0.050 mmol, 86% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 285H), 3.03 (m, 3H), 2.12 (m, 227H), 1.57 (m, 20H), 1.23 (m, 105H), 0.85 (t, *J* = 6.6 Hz, 29H). SEC: $M_w$ = 6.5 kDa, $M_n$ = 5.5 kDa, *Đ* = 1.14.

P(MeOx$_{91}$-*b*-NonOx$_{28}$-*b*-MeOx$_{91}$) (**10**) (Figure S23, S31)
To a flame dried microwave vial, MeCN (0.74 mL, anhydrous) and MeOx (250 μL, 0.250 g, 3.0 mmol, 90.0 equiv.)were added. After brief mixing, MeOTf (3.7 μL, 5.4 mg, 0.033 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 14 minutes, NonOx (193 μL, 193 mg, 0.975 mmol, 30 equiv.) was added under $N_2$ and heated to 140 °C. After 14 minutes, MeOx (250 μL, 0.250 g, 3.0 mmol, 90.0 equiv.) was added under $N_2$ and heated to 140 °C for 20 minutes, at which point the polymerization was quenched with MilliQ water (excess). The reaction mixture was evaporated to dryness to yield crude polymer (**10**) as a white solid. Polymer **10** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (355 mg, 0.017 mmol, 86% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.40 (m, 838H), 3.03 (m, 3H), 2.90-1.80 (m, 609H), 1.53 (m, 53H), 1.19 (m, 313H), 0.80 (m, 85H). SEC: $M_w$ = 13.8 kDa, $M_n$ = 11.0 kDa, *Đ* = 1.22.

P(EtOx$_{33}$-*b*-NonOx$_{11}$) (**11**) (Figure S24, S32)
To a flame dried microwave vial, MeCN (1.2 mL, anhydrous) and EtOx (300 μL, 0.300 g, 3.03 mmol, 30 equiv.) were added. After brief mixing, MeOTf (11.4 μL, 16.5 mg, 0.101 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 10 minutes, NonOx (200 μL, 200 mg, 1.01 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C for 7 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**11**) as a white solid. Polymer **11** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (300 mg, 0.054 mmol, 60% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 176H), 3.02 (m, 3H), 2.50-2.05 (m, 93H), 1.58 (m, 21H), 1.24 (m, 124H), 1.11 (m, 107H), 0.86 (t, *J* = 6.6 Hz, 30H). SEC: $M_w$ = 3.6 kDa, $M_n$ = 2.9 kDa, *Đ* = 1.23.

P(EtOx$_{93}$-*b*-NonOx$_{10}$) (**12**) (Figure S25, S32)
To a flame dried microwave vial, MeCN (1.3 mL, anhydrous) and EtOx (250 μL, 0.250 g, 2.52 mmol, 90 equiv.) were added. After brief mixing, MeOTf (3.2 μL, 4.6 mg, 0.028 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 34 minutes, NonOx (55 μL, 55 mg, 0.28 mmol, 10 equiv.) was added under $N_2$ and heated to 140 °C for 22 minutes, at

which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**12**) as a white solid. Polymer **12** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (300 mg, 0.020 mmol, 55% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 414H), 3.02 (m, 3H), 2.50-2.05 (m, 217H), 1.58 (m, 21H), 1.25 (m, 124H), 1.11 (m, 287H), 0.86 (t, $J$ = 6.6 Hz, 31H). SEC: $M_w$ = 8.0 kDa, $M_n$ = 5.3 kDa, $Đ$ = 1.35.

P($EtOx_{93}$-*b*-$NonOx_{29}$) (**13**) (Figure S26, S32)
To a flame dried microwave vial, MeCN (0.3 mL, anhydrous) and EtOx (200 μL, 0.200 g, 2.02 mmol, 90 equiv.) were added. After brief mixing, MeOTf (2.5 μL, 3.6 mg, 0.022 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 15 minutes, NonOx (133 μL, 133 mg, 0.68 mmol, 30 equiv.) was added under $N_2$ and heated to 140 °C for 12 minutes, at which point the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**13**) as a white solid. Polymer **13** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (274 mg, 0.018 mmol, 82% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.44 (m, 478H), 3.02 (m, 3H), 2.50-2.05 (m, 251H), 1.58 (m, 58H), 1.25 (m, 333H), 1.11 (m, 287H), 0.87 (t, $J$ = 6.6 Hz, 88H). SEC: $M_w$ = 7.8 kDa, $M_n$ = 6.2 kDa, $Đ$ = 1.21.

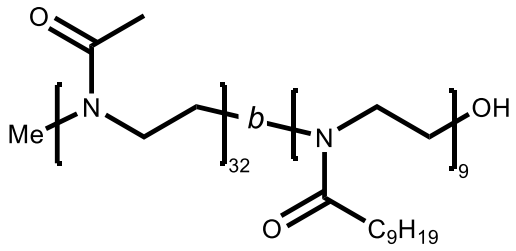
$PEG_{1K}$-*b*-$NonOx_{12}$ (**14**) (Figure S27, S33)
To a flame dried microwave vial, MeCN (0.5 mL, anhydrous) and NonOx (180 μL, 0.180 g, 0.91 mmol, 10.0 equiv.) were added. After brief mixing, $PEG_{1K}$-tosylate (100 mg, 0.09 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 26 minutes, the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**14**) as a white solid. Polymer **14** was purified by dissolving in DCM and washing against water, then further dialyzed against MeOH overnight, collected and evaporated to dryness (244 mg, 0.072 mmol, 87% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.80-3.20 (m, 122H), 3.37 (m, 3H), 2.36 (m, 26H), 1.59 (m, 25H), 1.25 (m, 151H), 0.86 (t, $J$ = 6.6 Hz, 37H). SEC: $M_w$ = 2.6 kDa, $M_n$ = 2.4 kDa, $Đ$ = 1.07.

$PEG_{2K}$-*b*-$NonOx_8$ (**15**) (Figure S28, S33)
To a flame dried microwave vial, MeCN (0.3 mL, anhydrous) and NonOx (209 μL, 0.209 g, 1.06 mmol, 10.0 equiv.) were added. After brief mixing, $PEG_{2K}$-tosylate (212 mg, 0.10 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 20 minutes, the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**15**) as a white solid. Polymer **15** was purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (278 mg, 0.078 mmol, 66% yield). $^1$H NMR (500 MHz, $CDCl_3$): δ 3.80-3.20 (m, 222H), 3.37 (m, 3H), 2.30 (m, 16H), 1.57 (m, 16H), 1.24 (m, 95H), 0.86 (t, $J$ = 6.6 Hz, 24H). SEC: $M_w$ = 4.2 kDa, $M_n$ = 3.9 kDa, $Đ$ = 1.05.

$PEG_{5K}$-*b*-$NonOx_8$ (**16**) (Figure S29, S33)
To a flame dried microwave vial, MeCN (0.3 mL, anhydrous) and NonOx (102 μL, 0.102 g, 0.52 mmol, 10.0 equiv.) were added. After brief mixing, $PEG_{5K}$-tosylate (258 mg, 0.05 mmol, 1.0 equiv.) was added and the mixture was heated at 140 °C in the microwave. After 50 minutes, the polymerization was quenched with MilliQ water (excess). After stirring overnight, the reaction mixture was evaporated to dryness to yield crude polymer (**16**) as a white solid. Polymer **16** was

purified by dialysis against 1:1 DCM:MeOH (vol%) overnight, collected and evaporated to dryness (280 mg, 0.043 mmol, 78% yield). $^{1}$H NMR (500 MHz, $CDCl_3$): δ 3.80-3.20 (m, 445H), 3.35 (m, 3H), 2.31 (m, 20H), 1.54 (m, 16H), 1.22 (m, 97H), 0.84 (m, 24H). SEC: $M_w$ = 8.5 kDa, $M_n$ = 8.3 kDa, $Đ$ = 1.05.

**$^1$H NMR Characterization**




P(MeOx$_{32}$-*b*-NonOx$_9$)
**5**

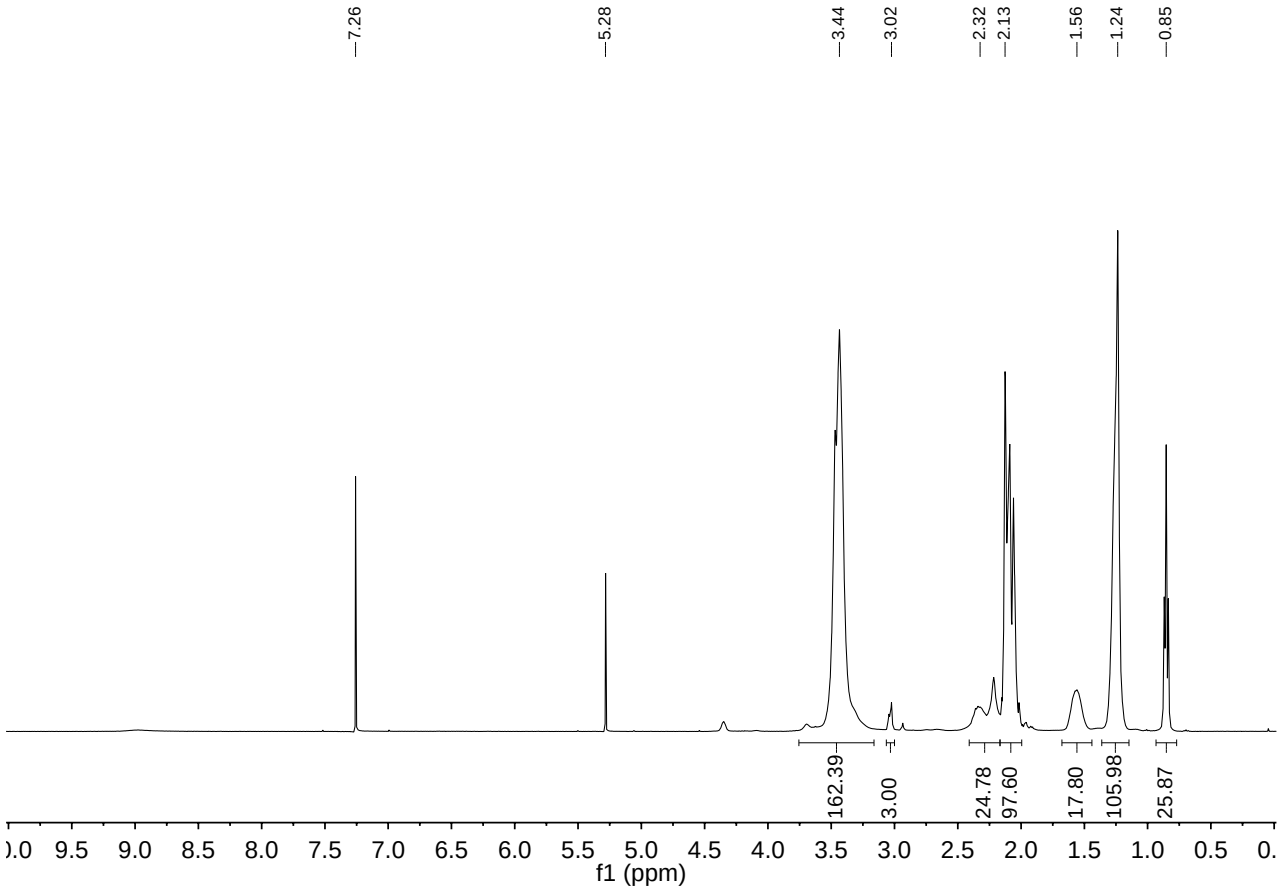
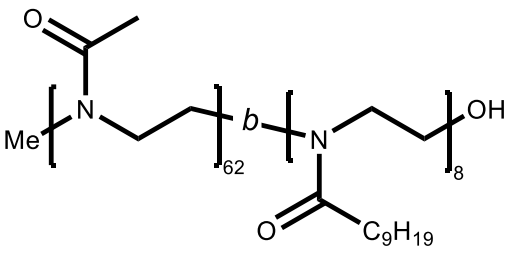



**Figure S18**. $^1$H NMR (500 MHz, $CDCl_3$) of copolymer **5** (MeOx$_{30}$-*b*-NonOx$_{10}$).




P(MeOx$_{62}$-*b*-NonOx$_8$)

**6**

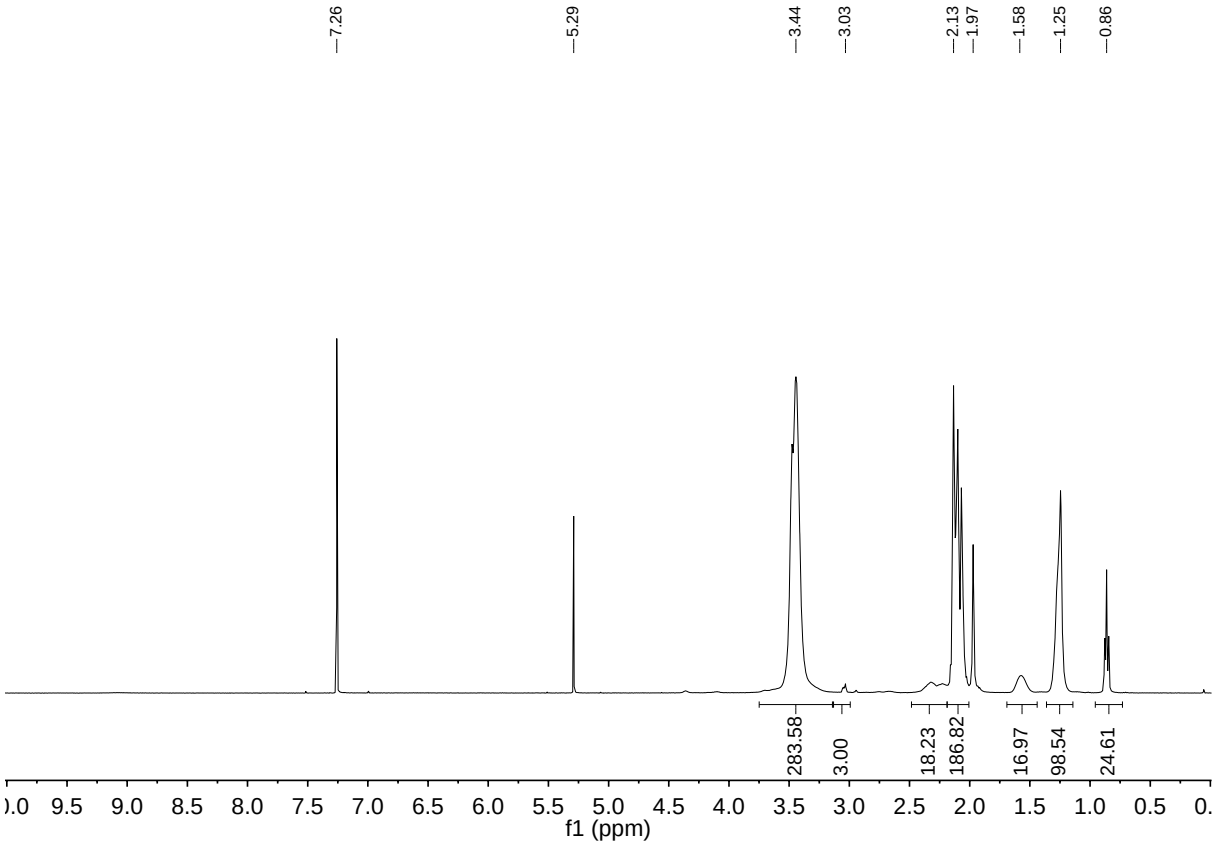



**Figure S19**. $^1$H NMR (500 MHz, $CDCl_3$) of copolymer **6** (MeOx$_{60}$-*b*-NonOx$_{10}$).

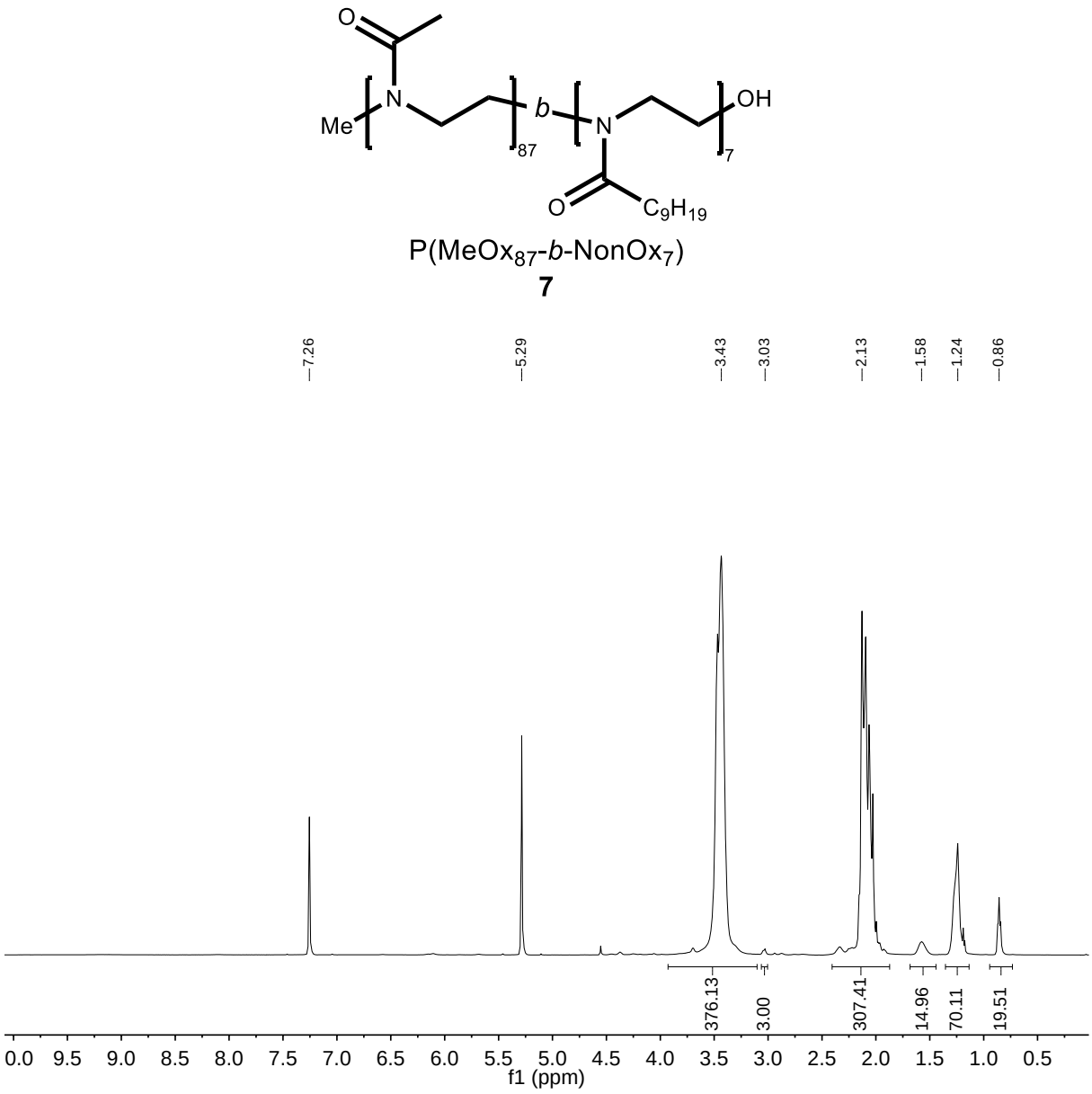
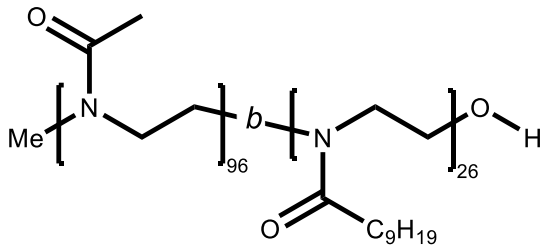



**Figure S20**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **7** ($MeOx_{90}$-*b*-$NonOx_{10}$).




P(MeOx$_{96}$-*b*-NonOx$_{26}$)

**8**

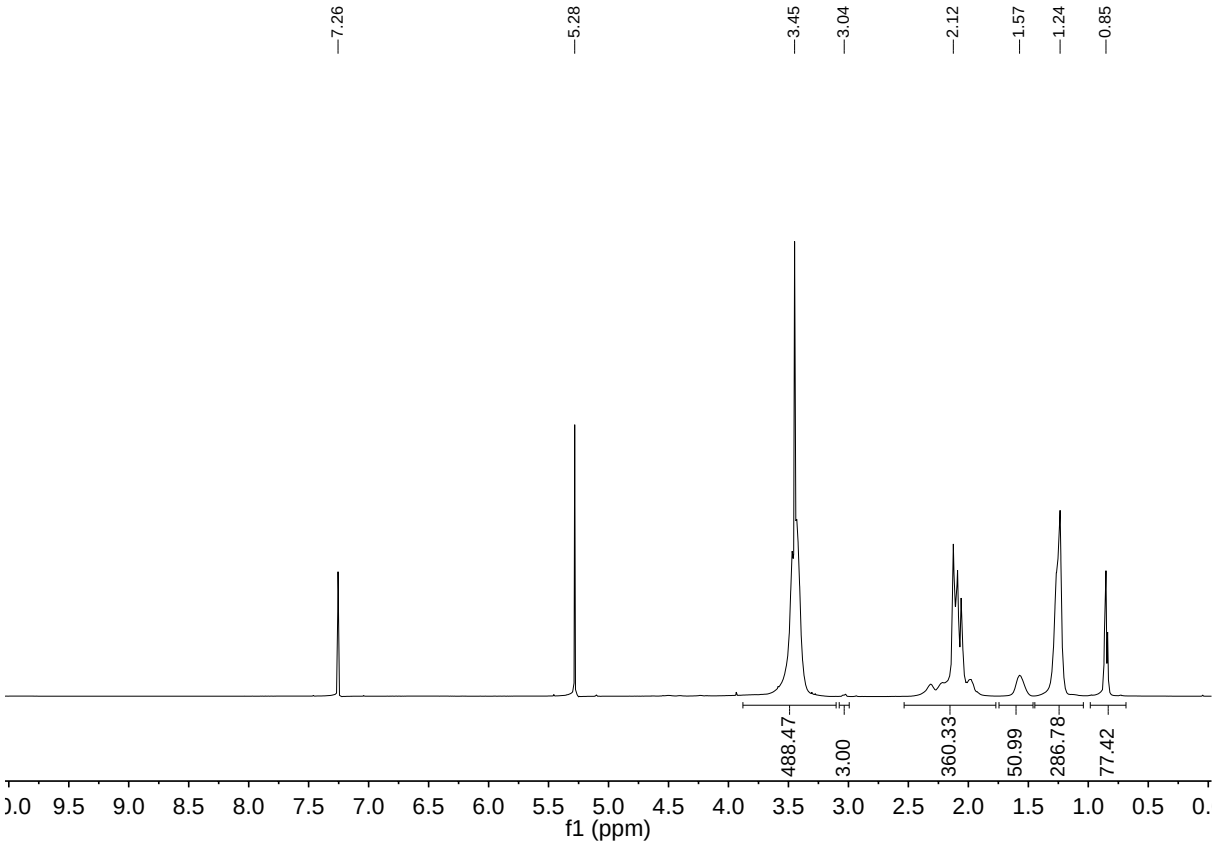
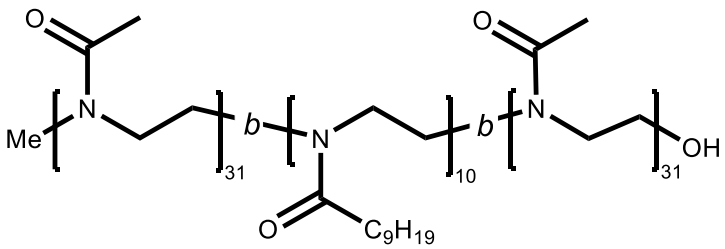



**Figure S21**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **8** (MeOx$_{90}$-*b*-NonOx$_{30}$).




$(MeOx_{31}\text{-}b\text{-}NonOx_{10}\text{-}b\text{-}MeOx_{31})$

**9**

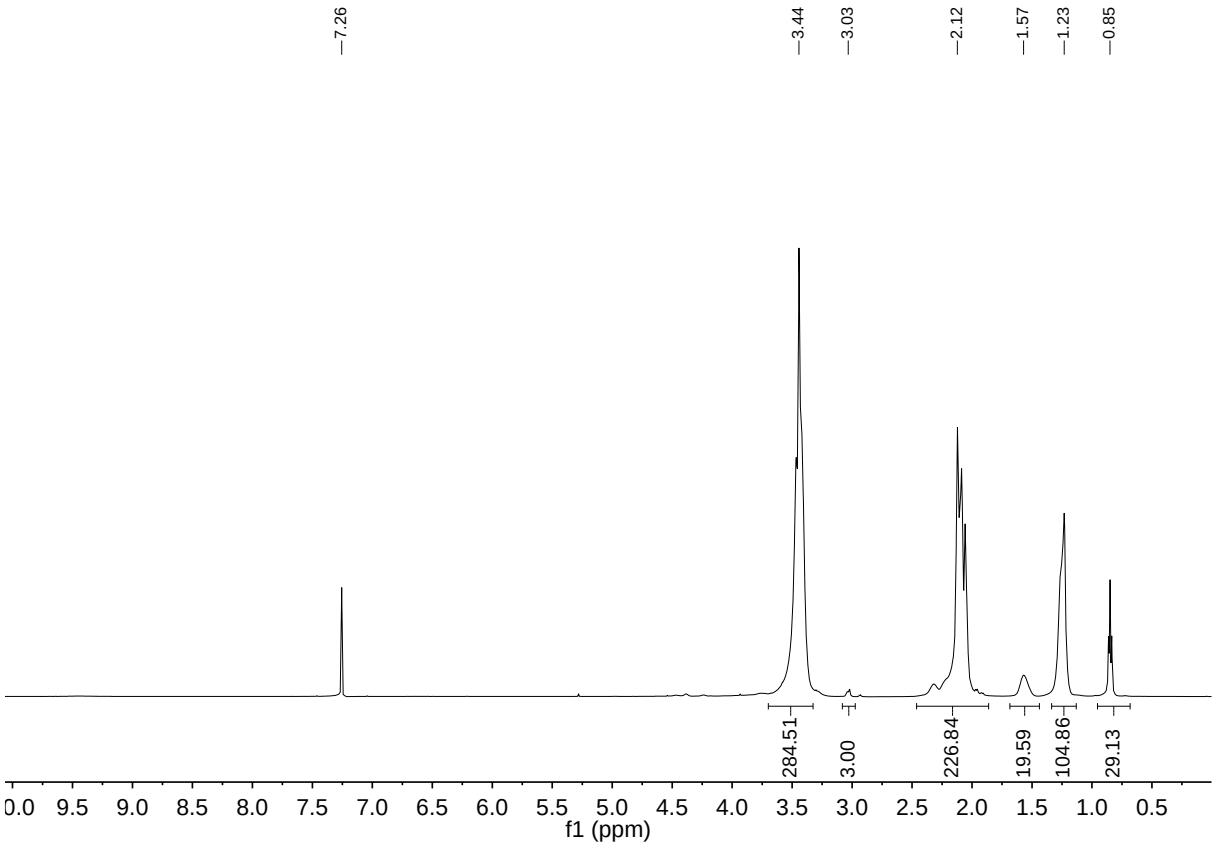
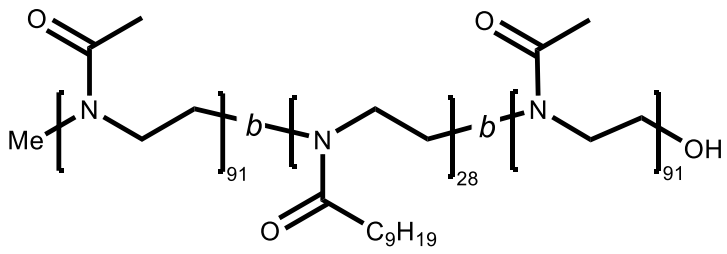



**Figure S22**. $^{1}H$ NMR (500 MHz, $CDCl_3$) of copolymer **9** ($MeOx_{30}$-*b*-$NonOx_{10}$-*b*-$MeOx_{30}$).




(MeOx$_{91}$-*b*-NonOx$_{28}$-*b*-MeOx$_{91}$)

**10**

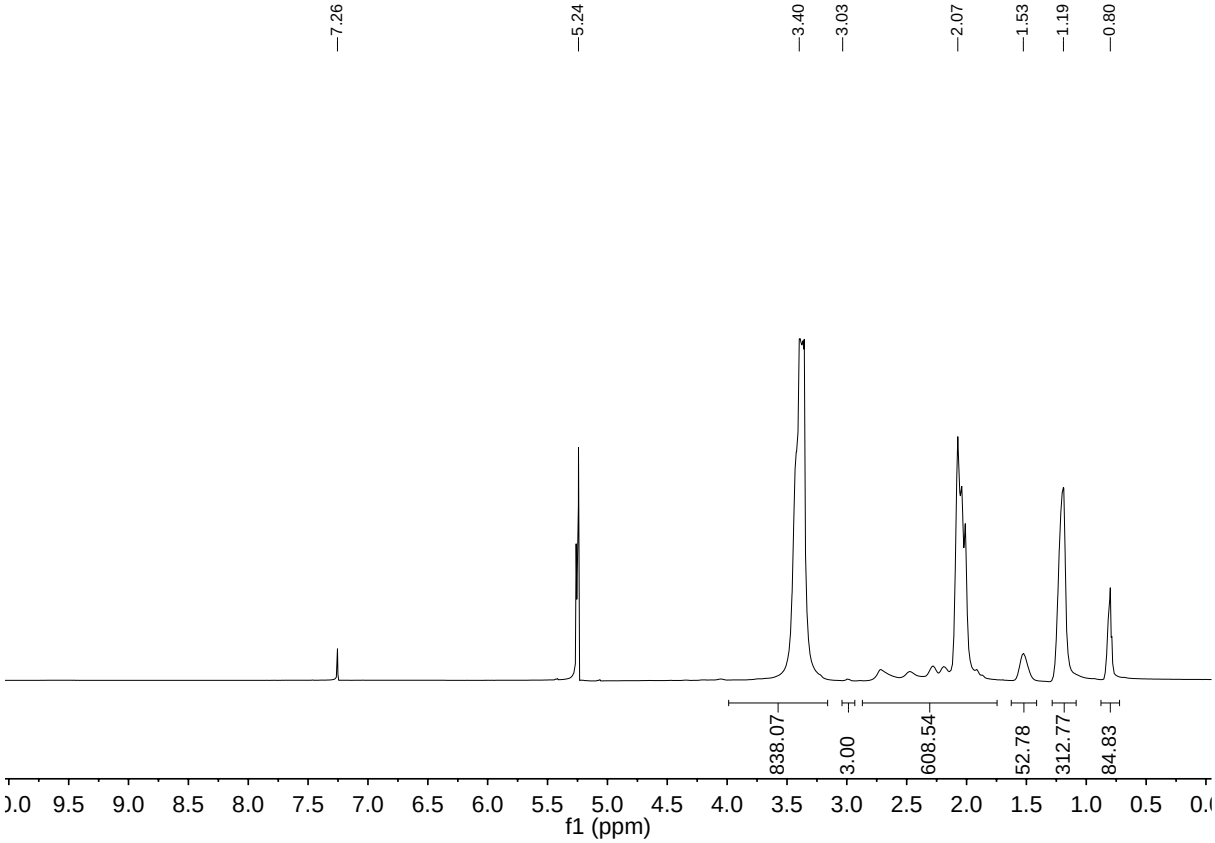
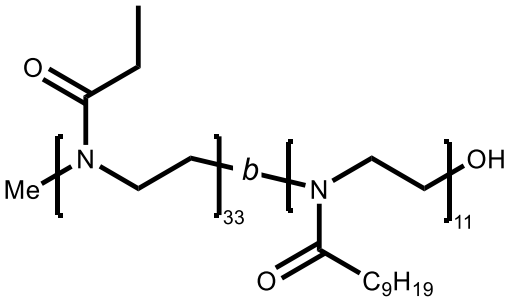



**Figure S23**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **10** (MeOx$_{90}$-*b*-NonOx$_{10}$-*b*-MeOx$_{90}$).




P(EtOx$_{30}$-*b*-NonOx$_{10}$)

**11**

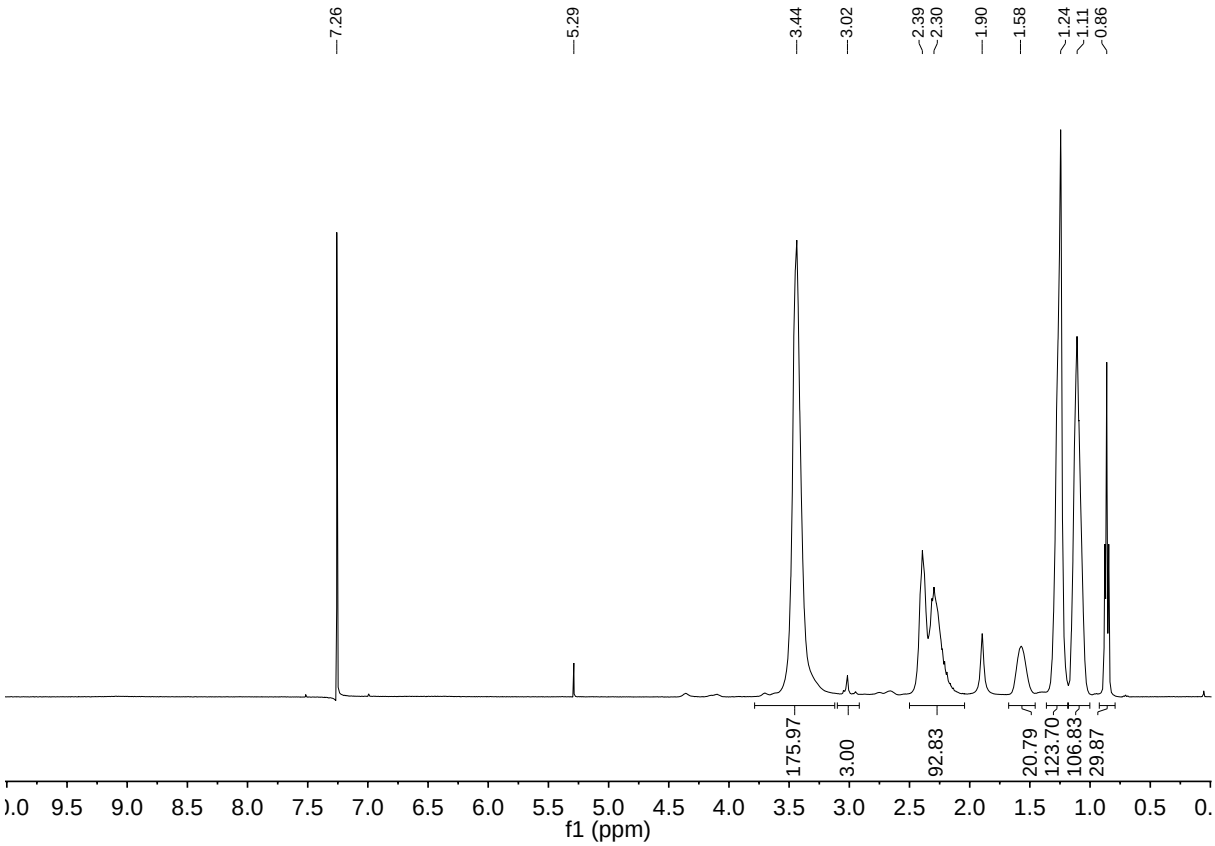



**Figure S24**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **11** (EtOx$_{30}$-*b*-NonOx$_{10}$).

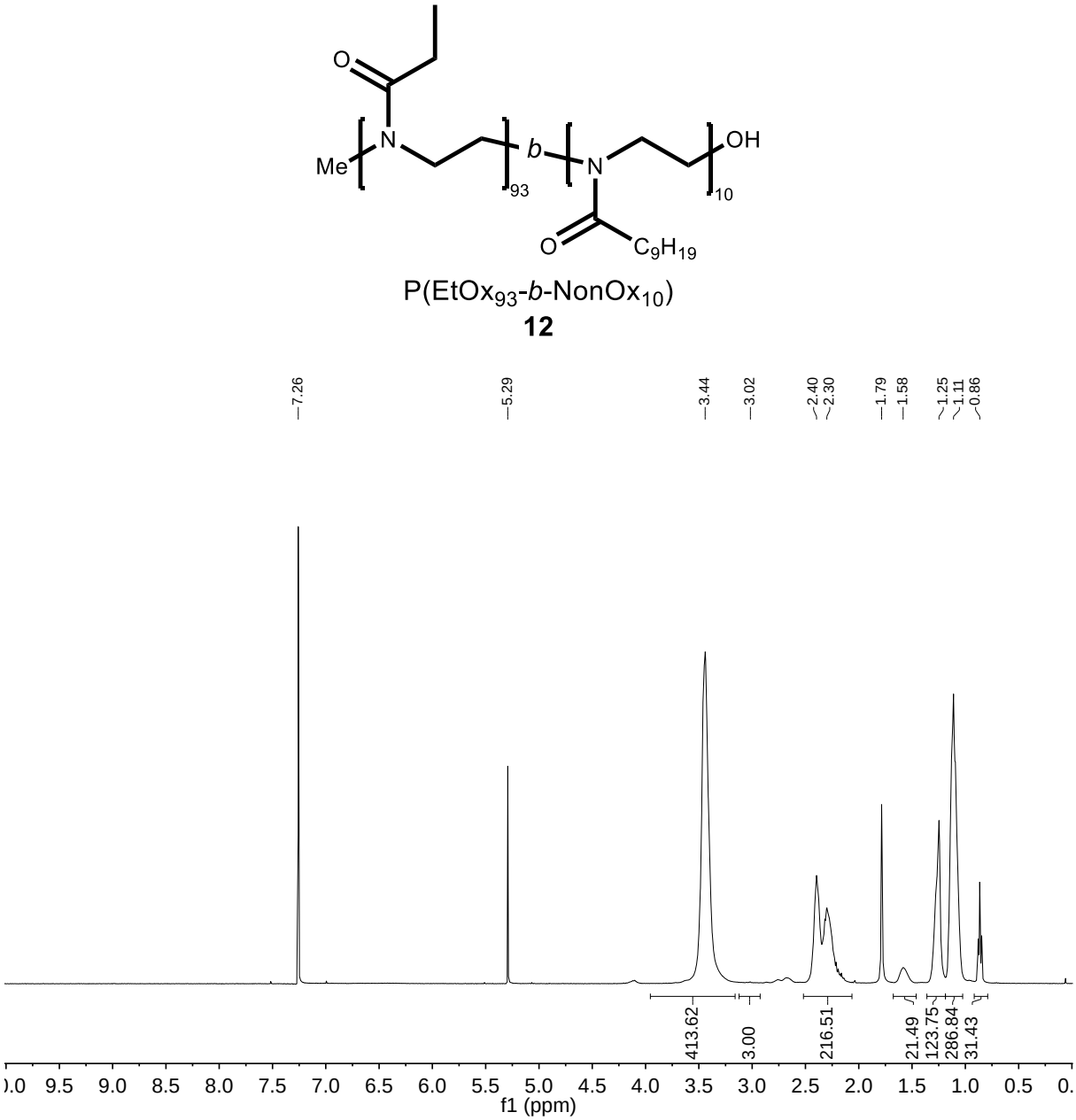
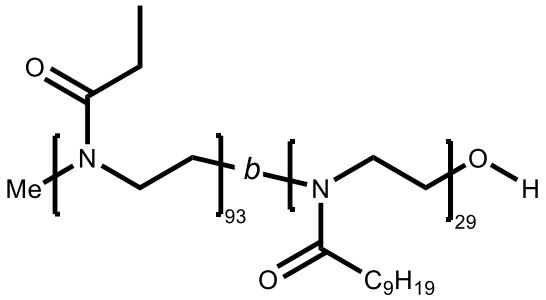



**Figure S25**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **12** ($EtOx_{90}$-*b*-$NonOx_{10}$).




P(EtOx$_{93}$-*b*-NonOx$_{29}$)

**13**

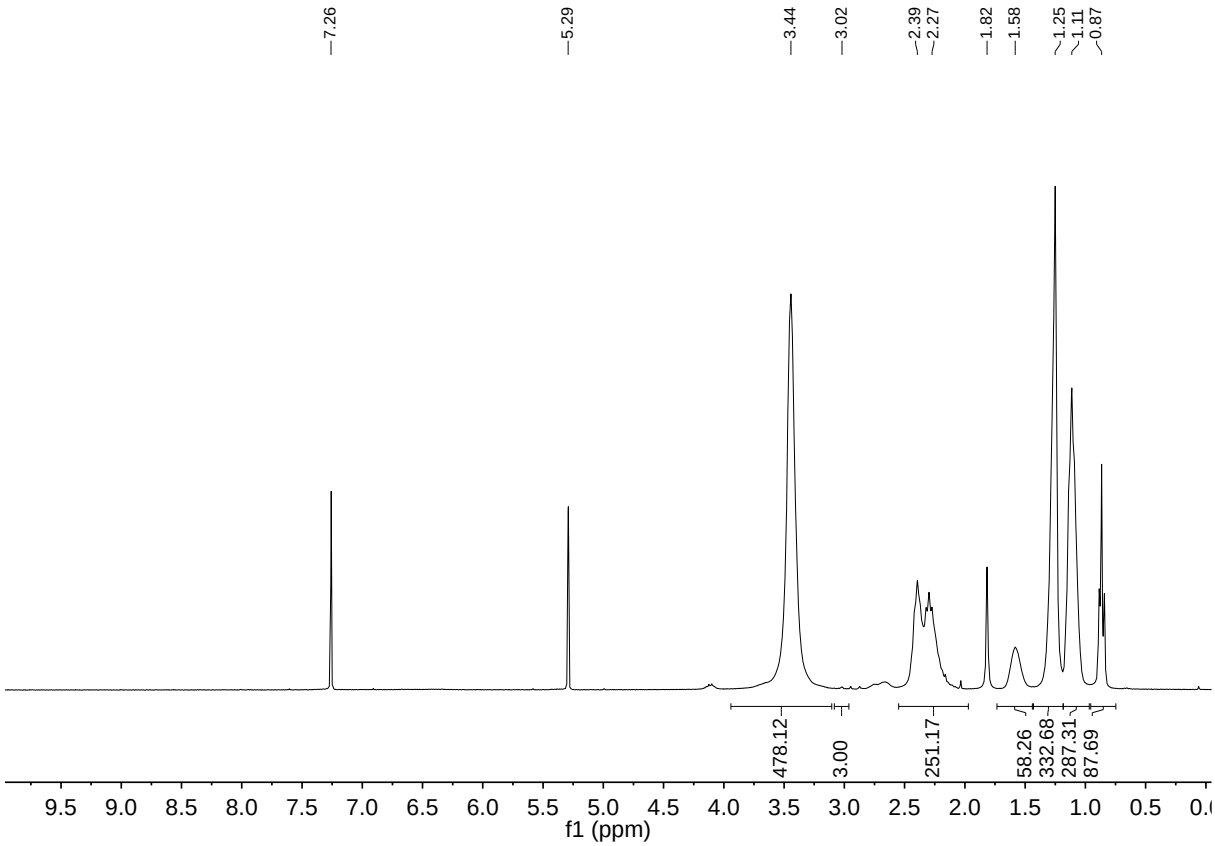
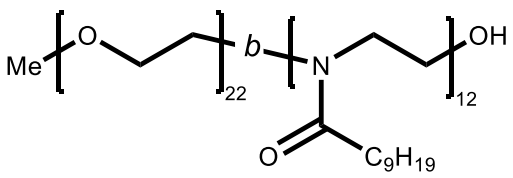



**Figure S26**. $^{1}$H NMR (500 MHz, $CDCl_3$) of copolymer **13** (EtOx$_{90}$-*b*-NonOx$_{30}$).




$PEG_{1K}$-*b*-$NonOx_{12}$

**14**

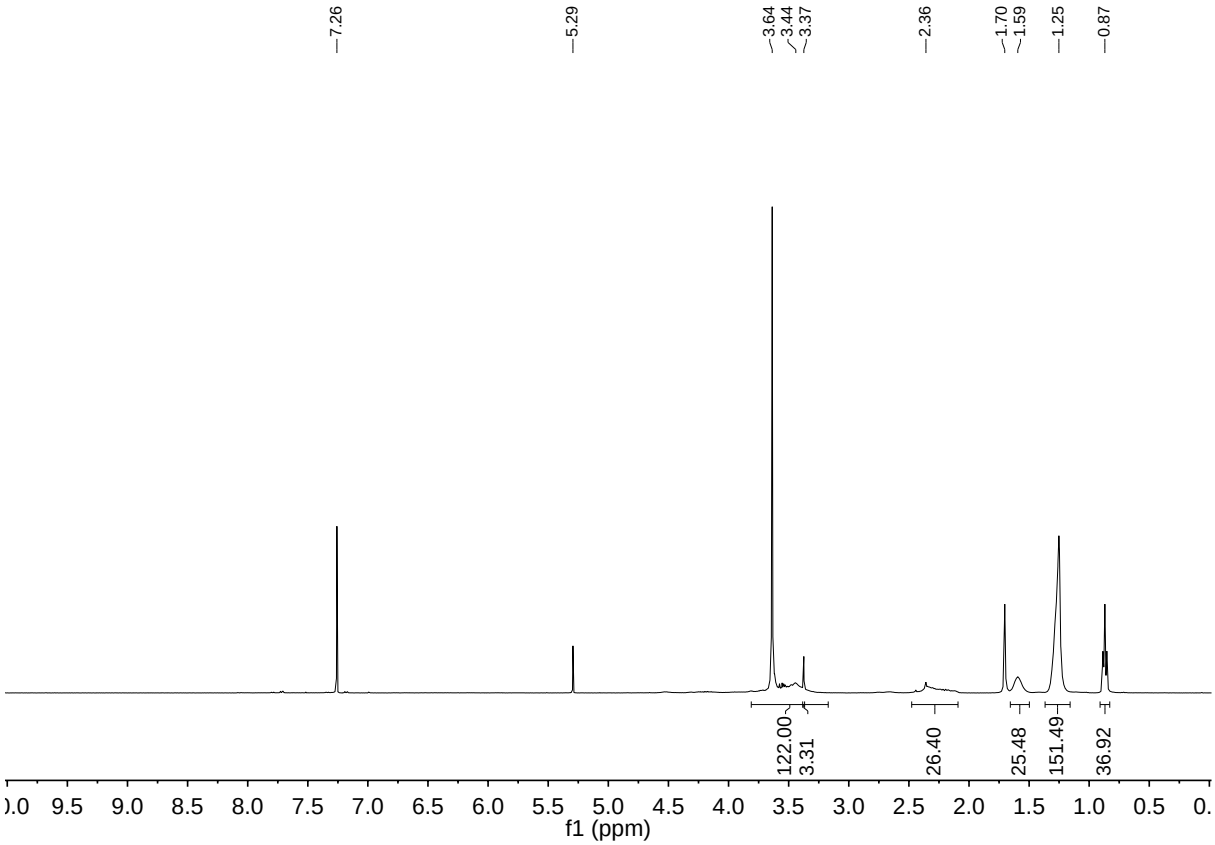
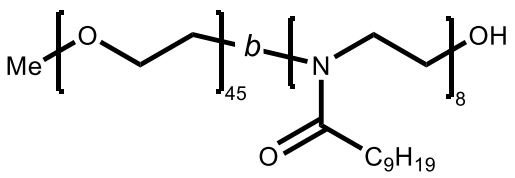



**Figure S27**. $^1$H NMR (500 MHz, $CDCl_3$) of copolymer **14** ($PEG_{1K}$-*b*-$NonOx_{10}$).




$PEG_{2K}$-*b*-$NonOx_8$

**15**

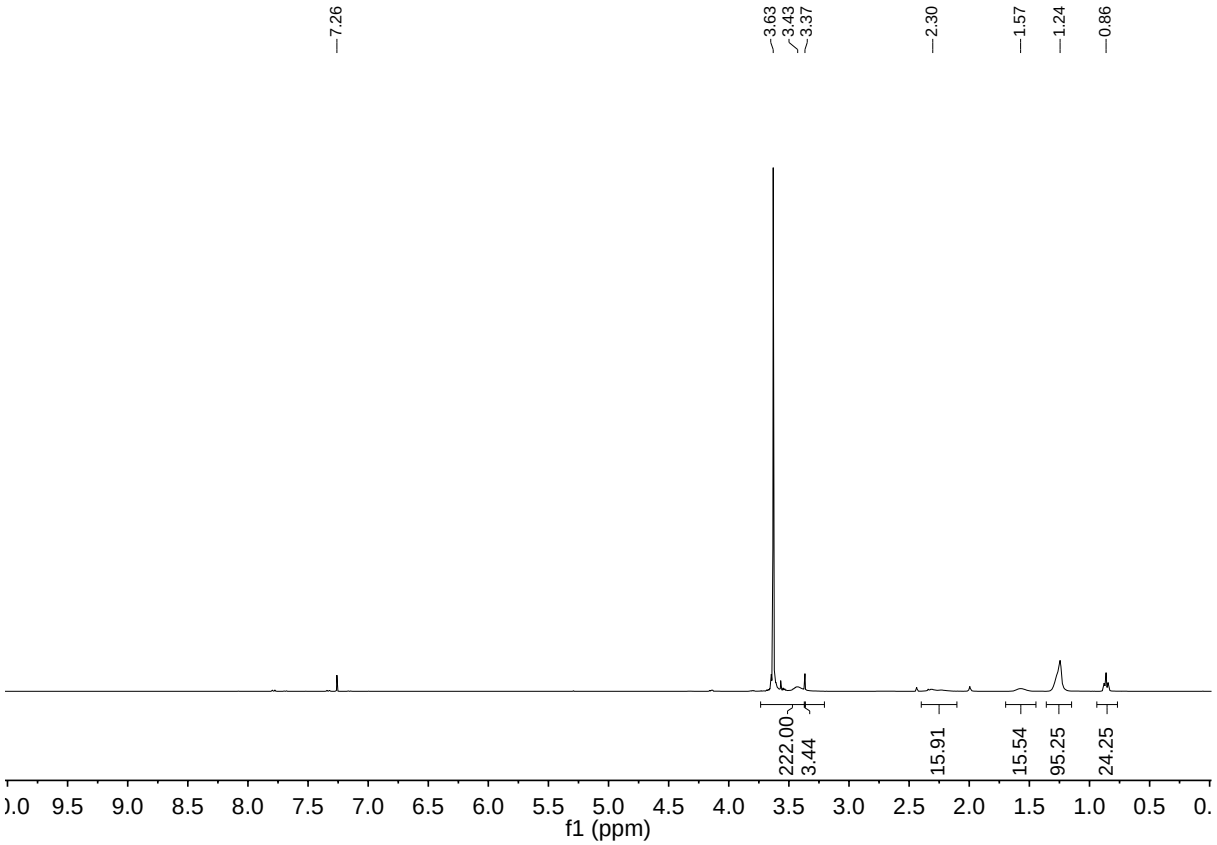
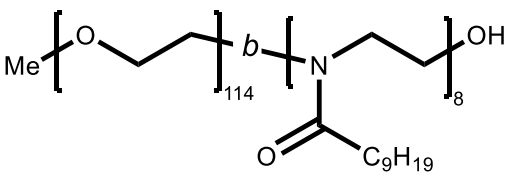



**Figure S28**. $^1$H NMR (500 MHz, $CDCl_3$) of copolymer **15** ($PEG_{2K}$-*b*-$NonOx_{10}$).




$PEG_{5K}$-*b*-$NonOx_8$

**16**

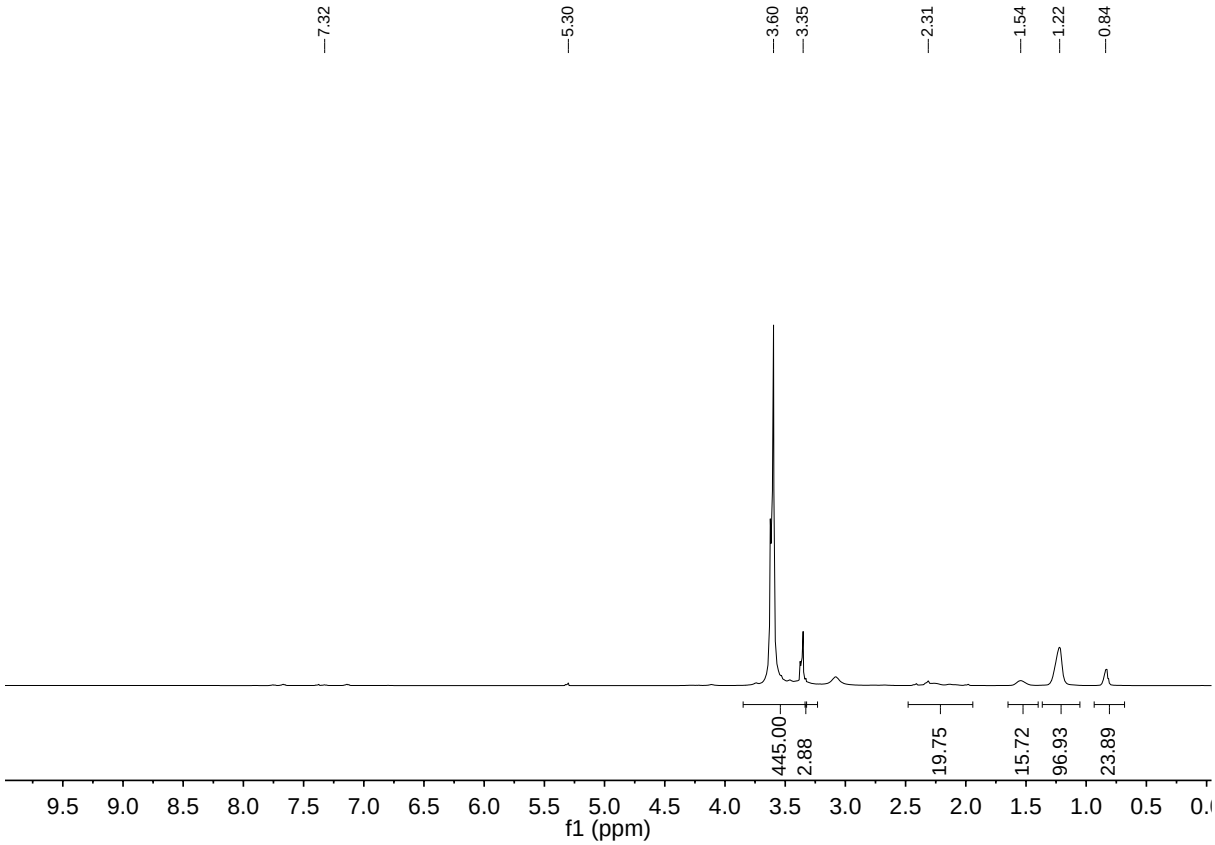



**Figure S29**. $^1$H NMR (500 MHz, $CDCl_3$) of copolymer **16** ($PEG_{5K}$-*b*-$NonOx_{10}$).

**SEC Traces**

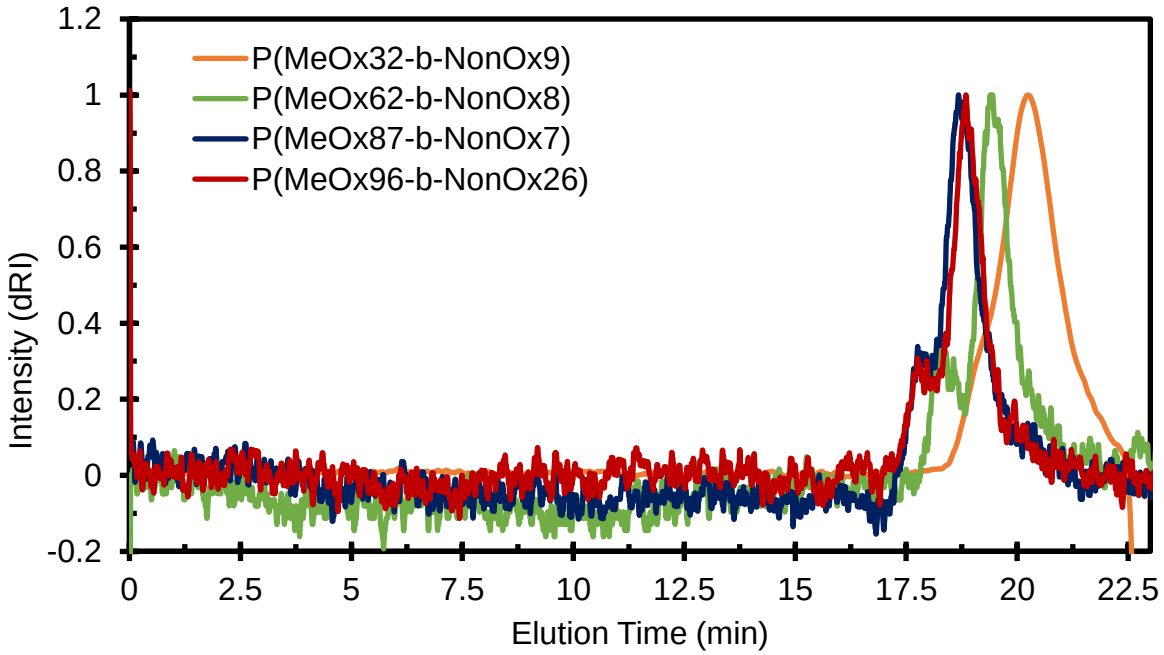



**Figure S30**. Size exclusion chromatogram of **5** (P(MeOx$_{30}$-*b*-NonOx$_{10}$)), **6** (P(MeOx$_{60}$-*b*-NonOx$_{10}$)), **7** (P(MeOx$_{90}$-*b*-NonOx$_{10}$)), **8** (P(MeOx$_{90}$-*b*-NonOx$_{10}$)). Eluent was DMF with LiBr (0.1 M) at 50 °C (flow rate: 0.80 mL/min).
Note: Shoulders have previously been observed in poly(2-oxazoline)s and may be attributed to aggregation, sample-column interactions,[9,10] or either extrinsic or intrinsic chain transfer/coupling side reactions that may occur at high monomer conversion and high reaction temperatures.[11,12]

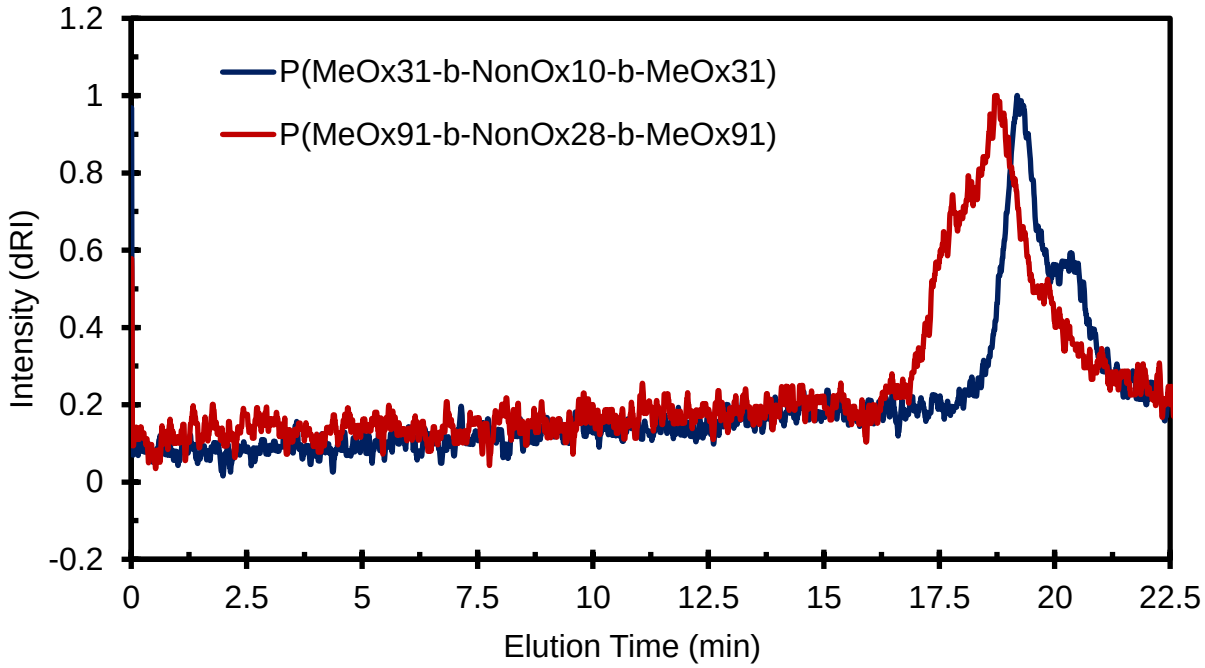



**Figure S31**. Size exclusion chromatogram of **9** (P(MeOx$_{30}$-*b*-Non$_{10}$-*b*-NonOx$_{30}$)), **10** (P(MeOx$_{90}$-*b*-Non$_{30}$-*b*-MeOx$_{90}$)). Eluent was DMF with LiBr (0.1 M) at 50 °C (flow rate: 0.80 mL/min). Note: Shoulders have previously been observed in poly(2-oxazoline)s and may be attributed to aggregation, sample-column interactions,[9,10] or either extrinsic or intrinsic chain transfer/coupling side reactions that may occur at high monomer conversion and high reaction temperatures.[11,12]

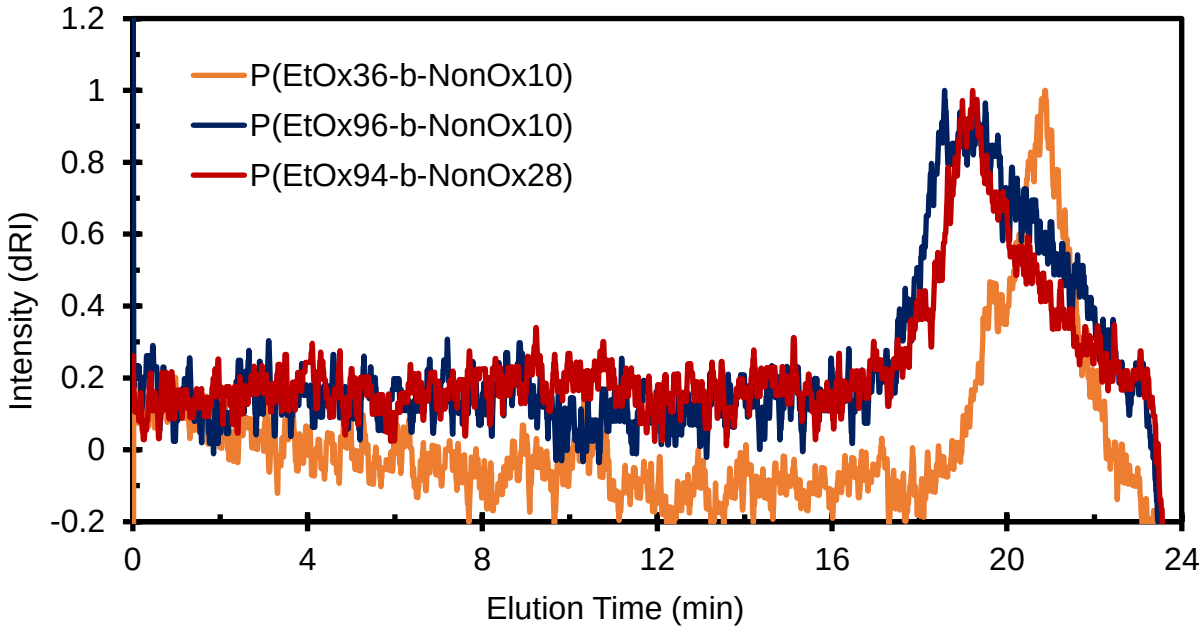



**Figure S32**. Size exclusion chromatogram of **11** (P(EtOx$_{30}$-*b*-NonOx$_{10}$)), **12** (P(EtOx$_{90}$-*b*-NonOx$_{10}$)), **13** (P(EtOx$_{90}$-*b*-NonOx$_{30}$)). Eluent was DMF with LiBr (0.1 M) at 50 °C (flow rate: 0.80 mL/min).
Note: Shoulders have previously been observed in poly(2-oxazoline)s and may be attributed to aggregation, sample-column interactions,[9,10] or either extrinsic or intrinsic chain transfer/coupling side reactions that may occur at high monomer conversion and high reaction temperatures.[11,12]

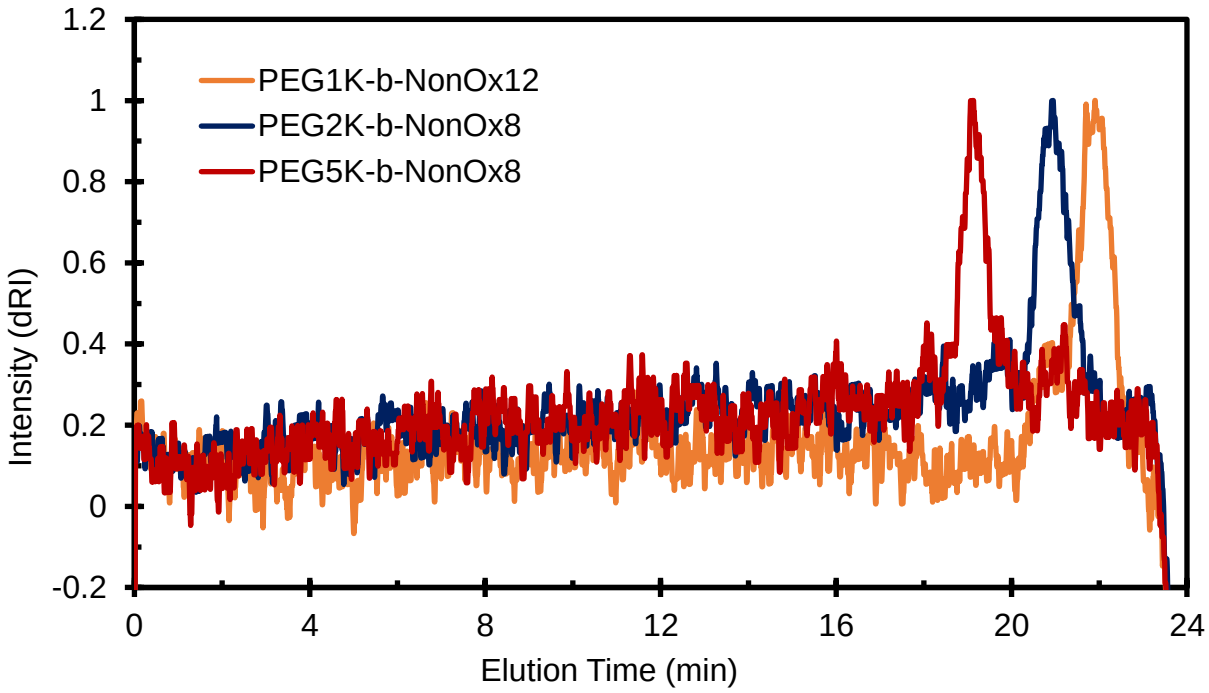



**Figure S33**. Size exclusion chromatogram of **14** ($PEG_{1K}$-*b*-$NonOx_{10}$), **15** ($PEG_{2K}$-*b*-$NonOx_{10}$), **16** ($PEG_{5K}$-*b*-$NonOx_{30}$). Eluent was DMF with LiBr (0.1 M) at 50 °C (flow rate: 0.80 mL/min).


(9) Luxenhofer, R.; Jordan, R. Click Chemistry with Poly(2-oxazoline)s. *Macromolecules*, **2006**, *39*, 3509–3516.

(10) R. Luxenhofer, TU München, **2007**.

(11) Wiesbrock, F.; Hoogenboom, R.; Leenen, M.; Van Nispen, S. F. G. M.; Van Der Loop, M.; Abeln, C. H.; Van Den Berg, A. M. J.; Schubert, U. S. Microwave-Assisted Synthesis of a 42-Membered Library of Diblock Copoly(2-oxazoline)s and Chain-Extended Homo Poly(2-oxazoline)s and Their Thermal Characterization. *Macromolecules*, **2005**, *38*, 7957–7966.

(12) Monnery, B. D.; Jerca, V. V.; Sedlacek, O.; Verbraeken, B.; Cavill, R.; Hoogenboom, R. Defined High Molar Mass Poly (2-Oxazoline)s. *Angew. Chem. Int. Ed.*, **2018**, *57*, 15400-15404.