Supporting Information

Synthesis of Customizable Macromolecular Conjugates as Building Blocks for Engineering Metal–Phenolic Network Capsules with Tailorable Properties

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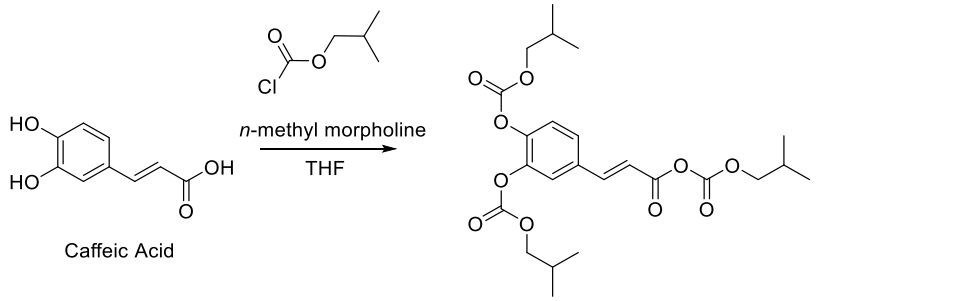
1. Materials and Instrumentation

The poly(ethylene glycol) (PEG) starting materials: 2-arm PEG-OH 600 Da was purchased from Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ, USA), 2-arm PEG-OH 2 kDa and 5 kDa were purchased from NanoCS (New York, NY, USA), (pentaerythritol core) 4-arm PEG-OH 5 kDa and 10 kDa, (hexaglycerol core) 8-arm PEG-OH 10 kDa and 20 kDa were purchased from Creative PEGWorks (Durham, NC, USA), and 2-arm PEG-NH_2 2.6 kDa was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Carboxylic acid-functionalized PS (PS-COOH, 1.86 ± 0.03 μm) particles were purchased from microParticles GmbH (Berlin, Germany). RAW 264.7 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). RAW 264.7 cells with low passage numbers from 20 to 30 were used in this study and all cells passed the mycoplasma test. Dulbecco's phosphate-buffered saline, Dulbecco's modified Eagle's medium (DMEM), wheat germ agglutinin Alexa Fluor 594 conjugate (WGA594), Hoechst 33342, and 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]2*H*-tetrazolium-5-carboxyanilide inner salt (XTT) were obtained from Life Technologies (Carlsbad, CA, USA). Caffeic acid, all other reagents and anhydrous solvents used for synthesis, iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), 3-(*N*-morpholino)propanesulfonic acid, tris-(hydroxymethyl)aminomethane (Tris), calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), sodium carbonate (Na_2CO_3), poly(sodium 4-styrenesulfonate) (PSS, 70 kDa), tetrahydrofuran (THF), ethylenediaminetetraacetic acid (EDTA), and fluorescein isothiocyanate-labeled dextran (FITC-dextran) with various average molecular weights (MW 4, 20, 59–77 (mean = 68), 250, 500, and 2000 kDa) were purchased from Sigma-Aldrich Chemical Company. Dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), petroleum benzine spirit 60−80 °C (HEX) and other solvents used for purifications and work-ups were of analytical grade and used directly without purification. Ultrapure water (18.2 MΩ cm), obtained from a three-stage Millipore Milli-Q plus 185 purification system (Millipore Corporation, Burlington, MA, USA), was used for all experiments. Thin layer chromatography was performed on an aluminum sheet coated with

silica gel 60 F254 (Merck) and visualization was performed under ultraviolet light. Silica gel flash column chromatography was conducted using a Reveleris flash chromatography system fitted with a 40 μm silica cartridge (Buchi). ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker UltraShield 400 MHz spectrometer (Bruker Biospin Corp., Germany) at 25 °C equipped with Bruker Topspin Software. Spectra were recorded for samples dissolved in deuterated solvent (chloroform, $CDCl_3$, or methanol, CD_3OD) and chemical shifts (δ) are reported as parts per million (ppm) from external tetramethylsilane. Fourier transform infrared (FTIR) spectra were recorded on a Tensor II FTIR spectrophotometer (Bruker, Germany). The hydrodynamic size was measured using a Zetasizer Nano-ZS (Malvern Instruments, UK). Differential interference contrast (DIC) and fluorescence microscopy analyses were performed on an inverted Olympus IX71 microscope (Tokyo, Japan). Scanning electron microscopy (SEM) was conducted on a FlexSEM microscope (Hitachi, Japan). Transmission electron microscopy (TEM) measurements were performed on an FEI Tecnai Spirit microscope (FEI Company, Hillsboro, OR, USA) operating at 120 kV to obtain standard TEM images and an FEI Tecnai F20 microscope (FEI Company, Hillsboro, OR, USA) operating at 200 kV to obtain high-angle annular dark-field (HAADF) images and energy-dispersive X-ray spectroscopy (EDX) mapping data. Atomic force microscopy (AFM) experiments were conducted using a JPK NanoWizard II BioAFM (JPK Instruments AG, Berlin, Germany) with tapping-mode cantilevers. Flow cytometry analysis was performed on an Apogee A50-Micro flow cytometer (Apogee Flow Systems, Hemel Hempstead, UK). Confocal laser scanning microscopy (CLSM) images were taken on a Nikon A1R+ confocal laser scanning microscope (Nikon Corporation, Tokyo, Japan).

2. Synthesis of (*E*)-3,4-Di-isobutoxycarbonyloxycinnamic mono-isobutyl carbonic anhydride (i-Boc-protected caffeic acid, iBocCAF)



(*E*)-3,4-Di-isobutoxycarbonyloxycinnamic mono-iso-butyl carbonic anhydride (iBoc-protected caffeic acid, **iBocCAF**)

Caffeic acid (2.5 g, 13.8 × 10^{-3} mol) was introduced into an oven-dried round bottom flask, followed by the addition of dry THF (50 mL) and isobutyl chloroformate (5.9 g, 5.6 mL, 43.5 × 10^{-3} mol). The solution was cooled using an ice/salt (−15°C) bath and kept under nitrogen. To the stirring solution was added *N*-methyl morpholine (94.8mL, 43.5 × 10^{-3} mol) dropwise after which the solution was left to stir at ambient temperature for 2 h. Hexane (20 mL) was then added to the reaction mixture and the solids were filtered out. The filtrate was evaporated and the resulting crude oil was purified by silica gel flash chromatography EtOAc/hexane (1:9→1:1 v/v) to afford the product, **iBocCAF**, as a clear oil (6.0 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.77 (1H, d, *J* = 16.0 Hz, ArCH=), 7.49 (1H, d, *J* = 2.0 Hz, ArH), 7.45 (1H, dd, *J* = 8.5, 2.0 Hz, ArH), 7.35 (1H, d, *J* = 8.5 Hz, ArH), 6.39 (1H, d, *J* = 16.0 Hz, =CH–), 4.09 (2H, d, *J* = 6.7 Hz, CH_2), 4.06 (2H, d, *J* = 6.7 Hz, CH_2, isobutyl), 4.05 (2H, d, *J* = 6.7 Hz, CH_2, isobutyl), 2.11–2.00 (3H, m, CH, isobutyl), 1.01–0.98 (18H, m, 6×CH_3, isobutyl). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 160.7 (C), 152.7 (C), 152.5 (C), 149.4 (C), 146.9 (CH), 144.7 (C), 143.1 (C), 132.6 (C), 127.1 (CH), 123.9 (CH), 122.9 (CH), 117.0 (CH), 75.7 (CH2), 75.5 (CH2), 27.9 (CH), 27.8 (CH), 18.9 (CH_3). Refer to Figure S1 for peak assignments.

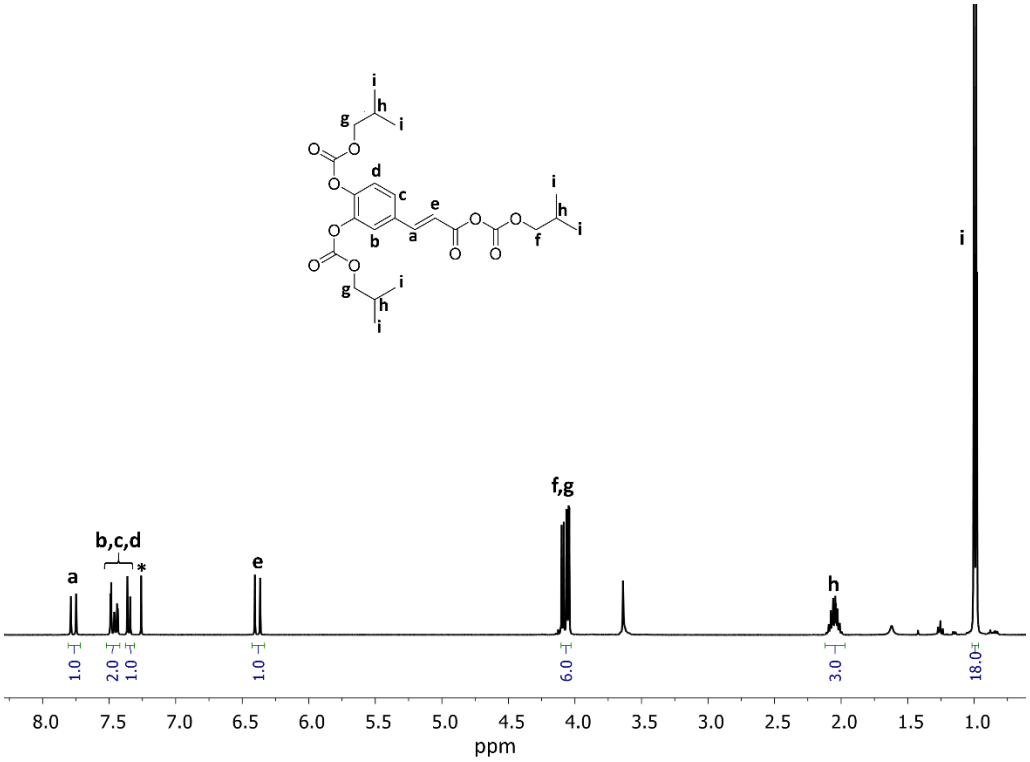
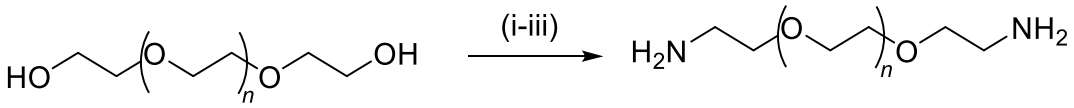


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of iBocCAF. *Residual NMR solvent peak.

3. Synthesis of NH_2-End-Functionalized Multi-Arm PEG Building Blocks ($[PEG_n\text{-}NH_2]_{2/4/8}$)

3.1. 2-Arm PEG-NH_2



3.1.1. 600 Da 2-Arm PEG-NH_2 ($[PEG_7\text{-}NH_2]_2$) from 2-Arm PEG-OH 600 Da

Step i: 2-Arm PEG-OH (600 Da, 4.0 g, 6.7×10^{-3} mol) and triethylamine (TEA; 1.8 g, 2.4 mL, 17.3×10^{-3} mol) were dissolved in dry DCM (18 mL) under nitrogen. The solution was cooled in an ice bath and then methane sulfonyl chloride, MesCl, (1.7 g, 1.1 ml, 14.7 mmol) was added dropwise. After the addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was then added to the residue and the white precipitate (residual salts) was retrieved by filtration. Toluene was then removed under reduced pressure and the product, **$[PEG_{\sim 6.5}\text{-Mesylate}]_2$**, **2-arm $PEG_{\sim 13}$-Mesylate**, was isolated as a low melting point waxy solid (4.7 g, yield: quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.08 (s,

2×C*H*$_3$−SO$_3$−, 6H), 3.63 (m, –CH$_2$CH$_2$–O– of PEG, 13 EG; 2 × 6.5 EG), 4.36 (t, *J* = 4.5 Hz, 2×CH$_3$SO$_3$−C*H*$_2$−, 4H).

Step ii: The above product **2-arm PEG$_{13}$-Mesylate** (4.7 g, 6.4 × 10^{-3} mol) was dissolved in dimethylformamide (DMF; 30 mL) followed by the addition of sodium azide (2.0 g, 0.013 mol), and the solution was then heated to 75 °C for 16 h. The majority of the DMF was evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in EtOAc, cooled and filtered, and then the filtrate was further concentrated under vacuum. The product, **[PEG$_{\sim 7.5}$-N$_3$]$_2$**, **2-arm PEG$_{\sim 15}$-Azide**, was isolated as a light brown, low melting point, waxy solid (4.0 g, yield: ~90%). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 3.36 (t, *J* = 4.9 Hz, 2×N$_3$−C*H*$_2$−, 4H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 15 EG, 2 × 7.5 EG).

Step iii: The above product **2-arm PEG$_{\sim 15}$-Azide** (4.0 g, 5.7 × 10^{-3} mol) was dissolved in THF (30 mL) and added to a solution of triphenylphosphine (PPh$_3$; 3.5 g, 13.3 mmol) in THF (80 mL). The solution was stirred at room temperature for 16 h under N$_2$. Water (5 mL) was then added and the mixture was then allowed to stir at room temperature for a further 5 h. THF was then removed under reduced pressure and the mixture was diluted with water (30 mL). The mixture was then washed 3× with EtOAc (to remove the PPh$_3$O) and the aqueous layer was then freeze-dried to remove water. The product, **[PEG$_{\sim 7}$-NH$_2$]$_2$**, **2-arm PEG$_{\sim 14}$-NH$_2$**, was isolated as a pale yellow, low melting point solid (3.2 g, yield: ~80 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 2.82 (t, *J* = 5.3 Hz, 2×NH$_2$−C*H*$_2$−, 4H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 14 EG, 2 × 7 EG). Refer to Figure S2 for peak assignments.

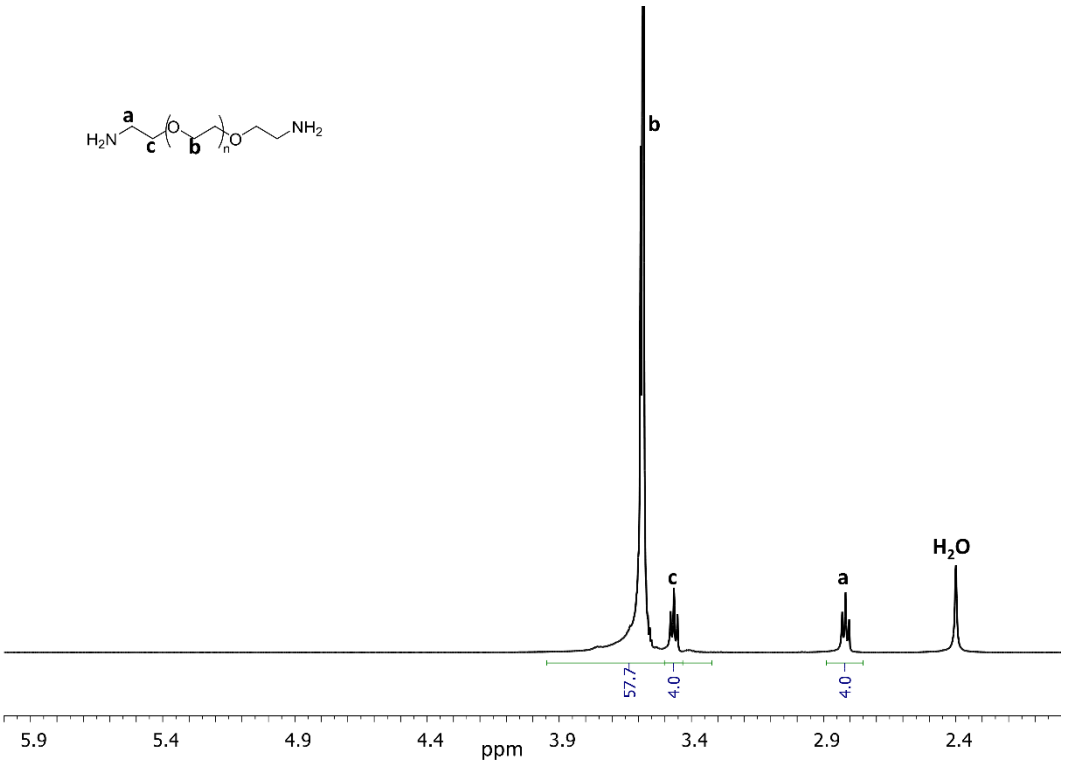


Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of $[PEG_{\sim 7}\text{-}NH_2]_2$, 2-arm $PEG_{\sim 14}\text{-}NH_2$.

3.1.2. 2.5 kDa 2-Arm PEG-NH_2 ($[PEG_{\sim 28}\text{-}NH_2]_2$) from 2-Arm PEG-OH 2 kDa

Step i: 2-Arm PEG-OH (2 kDa, 2.0 g, 1.0 mmol) and TEA (607 mg, 836 µL, 6.0×10^{-3} mol) were dissolved in dry DCM (15 mL) under nitrogen. The solution was cooled in an ice bath and MesCl, (573 mg, 390 µL, 5.0×10^{-3} mol) was then added dropwise. After the addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was added to the residue and the white precipitate (residual salt) was removed by filtration. Toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was added dropwise to excess cold diethyl ether to precipitate the product, **$[PEG_{\sim 25.5}\text{-Mesylate}]_2$**, **2-arm $PEG_{\sim 51}$-Mesylate**, as an off-white powder (1.9 g, yield: ~95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.08 (s, 2 × C*H*$_3$–SO_3–, 6H), 3.63 (m, –CH_2CH_2–O– of PEG, 51 EG; 2 × 25.5 EG), 4.37 (t, *J* = 4.5 Hz, 2 × CH_3SO_3–C*H*$_2$–, 4H).

Step ii: The above product **2-arm $PEG_{\sim 51}$-Mesylate** (1.9 g, 8.6×10^{-4} mol) was dissolved in DMF (10 mL) followed by the addition of sodium azide (0.28 g, 4.3 mmol) and the solution was then heated

to 75 °C for 16 h. DMF was mostly evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled and filtered, and the concentrated filtrate was then precipitated into cold diethyl ether. The product, **$[PEG_{\sim 26}\text{-}N_3]_2$**, **2-arm $PEG_{\sim 52}$-Azide**, was isolated as an off-white powder (1.3 g, yield: ~70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.38 (t, *J* = 4.9 Hz, 2 × N_3–C*H*$_2$–, 4H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 52 EG, 2 × 26 EG).

Step iii: The above product **2-arm $PEG_{\sim 52}$-Azide** (1.3 g, 5.9×10^{-4} mol) was dissolved in MeOH (12 mL) followed by the addition of PPh_3 (0.53 g, 2.4×10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **$[PEG_{\sim 28}\text{-}NH_2]_2$**, **2-arm $PEG_{\sim 55}$-NH_2**, as an off-white powder (1.2 g, yield: ~92%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.90 (t, *J* = 5.3 Hz, 2 × NH_2–C*H*$_2$–, 4H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 55 EG, 2 × 27.5 EG) ppm. Refer to Figure S3 for peak assignments.

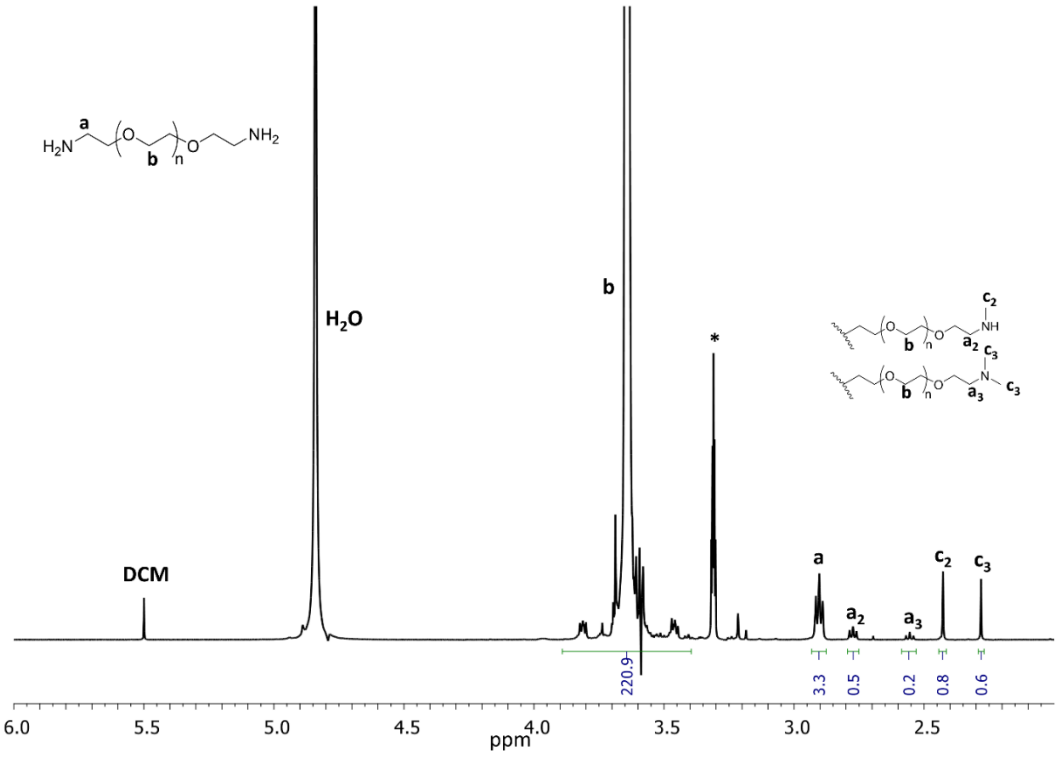


Figure S3. ^{1}H NMR (400 MHz, CD_3OD) spectrum of $[PEG_{\sim 28}\text{-}NH_2]_2$, 2-arm $PEG_{\sim 55}$-NH_2. Minor amounts of *N*-methyl amine arms formed, as evidenced by additional triplets from CH_2 protons α to nitrogen (a_2 and a_3 peaks) and the corresponding methyl singlets (c_2 and c_3 peaks). *Residual NMR solvent peak.

3.1.3. 5 kDa 2-Arm PEG-NH_2 ($[PEG_{\sim 59}\text{-}NH_2]_2$) from 2-Arm PEG-OH 5 kDa

Step i: 2-Arm PEG-OH (5 kDa, 3.0 g, 6.0×10^{-4} mol) and TEA (364 mg, 500 µL, 3.6×10^{-3} mol) were dissolved in dry DCM (15 mL) under nitrogen. The solution was cooled in an ice bath and MesCl (343 mg, 230 µL, 3.0 mmol) was then added dropwise. After addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was then added to the residue and the white precipitate (residual salts) was removed by filtration. Toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was dropped into excess cold diethyl ether to precipitate the product, **$[PEG_{\sim 60}\text{-Mesylate}]_2$**, **2-arm $PEG_{\sim 120}$-Mesylate**, as an off-white powder (2.9 g, yield: ~95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.08 (s, 2 × C*H*$_3$−SO_3−, 6H), 3.63 (m, –CH_2CH_2–O– of PEG, 120 EG; 2 × 60 EG), 4.37 (t, *J* = 4.5 Hz, 2 × CH_3SO_3−C*H*$_2$−, 4H).

Step ii: The above product **2-arm $PEG_{\sim 120}$-Mesylate** (2.8 g, 5.6×10^{-4} mol) was dissolved in DMF (10 mL) followed by the addition of sodium azide (0.18 g, 2.8×10^{-3} mol) and the solution was then heated to 75 °C for 16 h. DMF was mostly evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled, and filtered, and then the concentrated filtrate was precipitated into cold diethyl ether. The product, **$[PEG_{\sim 55}\text{-}N_3]_2$**, **2-arm $PEG_{\sim 110}$-Azide**, was isolated as an off-white powder (2.6 g, yield: ~90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.38 (t, *J* = 4.9 Hz, 2 × N_3−C*H*$_2$−, 4H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 110 EG, 2 × 55 EG).

Step iii: The above product **2-arm $PEG_{\sim 110}$-Azide** (2.1 g, 4.2×10^{-4} mol) was dissolved in MeOH (20 mL) followed by the addition of PPh_3 (0.38 g, 1.7×10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **$[PEG_{\sim 59}\text{-}NH_2]_2$**, **2-arm $PEG_{\sim 118}$-NH_2**, as an off-white powder (1.9 g, yield: ~90 %). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.86 (t, *J* = 5.3 Hz,

2×NH_2−CH_2−, 4H), 3.63 (m, –CH_2CH_2–O– of PEG, 118 EG, 2 × 59 EG). Refer to Figure S4 for peak assignments.

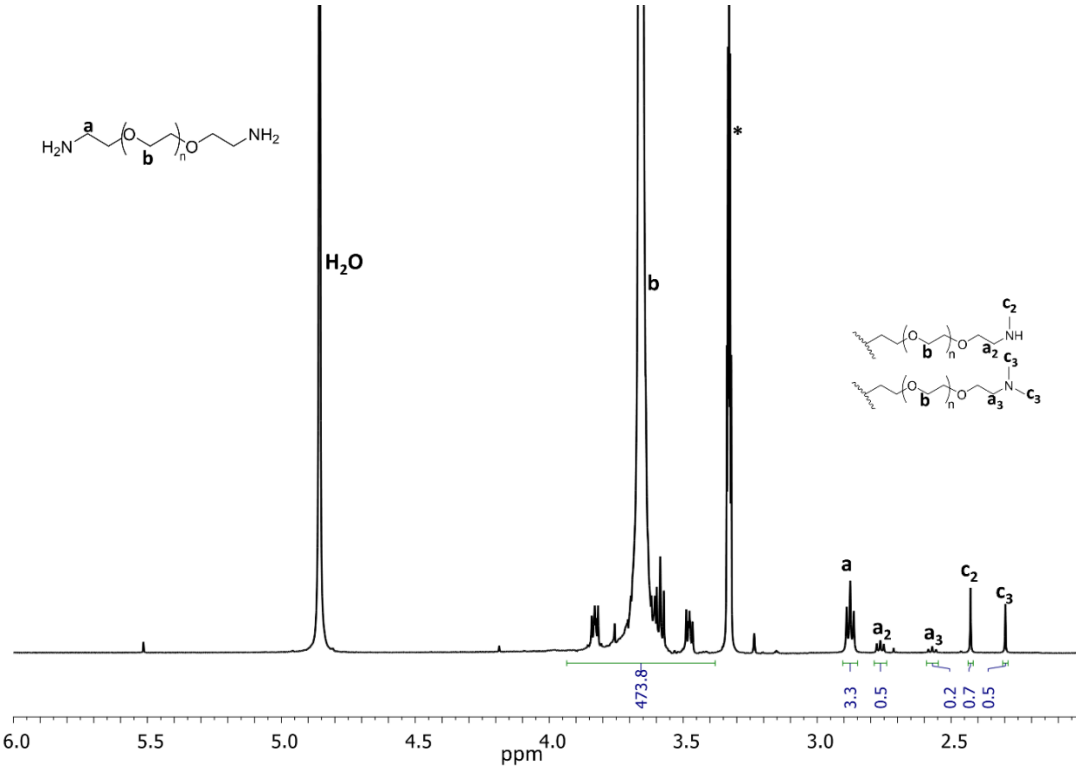
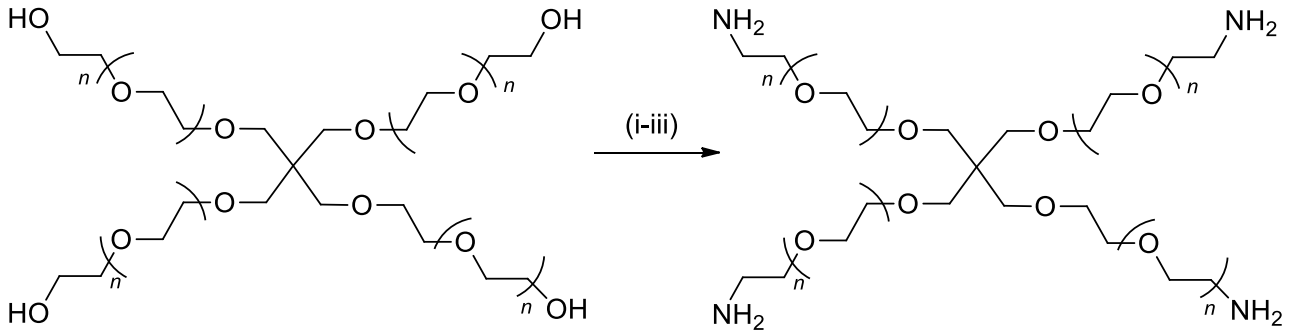


Figure S4. ^{1}H NMR (400 MHz, CD_3OD) spectrum of [PEG$_{\sim 59}$-NH_2]$_2$, 2-arm PEG$_{\sim 118}$-NH_2. Minor amounts of *N*-methyl amine arms formed, as evidenced by additional triplets from CH_2 protons α to nitrogen (a_2 and a_3 peaks) and the corresponding methyl singlets (c_2 and c_3 peaks). *Residual NMR solvent peak.

3.2. 4-Arm PEG-NH_2



n = ~30 for 5 kDa and *n* = ~60 for 10 kDa

3.2.1. 5 kDa 4-Arm PEG-NH_2 ([PEG$_{\sim 32}$-NH_2]$_4$) from 4-Arm PEG-OH 5 kDa

Step i: 4-Arm PEG-OH (5 kDa, 3.0 g, 6 × 10^{-4} mol) and TEA (730 mg, 1.0 mL, 7.2 × 10^{-3} mol) were dissolved in dry DCM (25 mL) under nitrogen. The solution was cooled in an ice bath and MesCl

(687 mg, 464 µL, 6.0×10^{-3} mol) was then added dropwise. After the addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was added to the residue and the white precipitate (residual salt) was removed by filtration. The toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was dropped into excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 30}$-Mesylate]$_4$**, **4-arm PEG$_{\sim 120}$-Mesylate**, as an off-white powder (2.9 g, yield: ~95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.05 (s, 4 × C*H*$_3$−SO$_3$−, 12H), 3.38 (s, 4 × CH_2O from pentaerythritol core, 8H), 3.63 (m, –CH_2CH_2–O– of PEG, 120 EG; 4 × 30 EG), 4.35 (m, 4 × CH_3SO_3−C*H*$_2$−, 8H).

Step ii: The above product **4-arm PEG$_{\sim 120}$-Mesylate** (2.8 g, 5.6×10^{-4} mol) was dissolved in DMF (12 mL) followed by the addition of sodium azide (0.36 g, 5.6×10^{-3} mol) and the solution was then heated to 75 °C for 16 h. The majority of the DMF was evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled, and filtered, and then the concentrated filtrate was precipitated into cold diethyl ether. The product, **[PEG$_{\sim 30}$-N$_3$]$_4$**, **4-arm PEG$_{\sim 120}$-Azide**, was isolated as an off-white powder (2.6 g, yield: ~90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.37 (t, *J* = 4.9 Hz, 4 × N$_3$−C*H*$_2$−, 8H), 3.40 (s, 4 × CH_2O from pentaerythritol core, 8H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 120 EG, 4 × 30 EG).

Step iii: The above product **4-arm PEG$_{\sim 120}$-Azide** (2.0 g, 4.0×10^{-4} mol) was dissolved in MeOH (12 mL) followed by the addition of PPh_3 (1.05 g, 4.0×10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **[PEG$_{\sim 32}$-NH$_2$]$_4$**, **4-arm PEG$_{\sim 128}$-NH$_2$**, as an off-white powder (1.9 g, yield: ~90 %). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.89 (t, *J* = 5.3 Hz, 4 × NH_2−C*H*$_2$−, 8H), 3.40 (s, 4 × CH_2O from pentaerythritol core, 8H), 3.45 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 128 EG, 4 × 32 EG). Refer to Figure S5 for peak assignments.

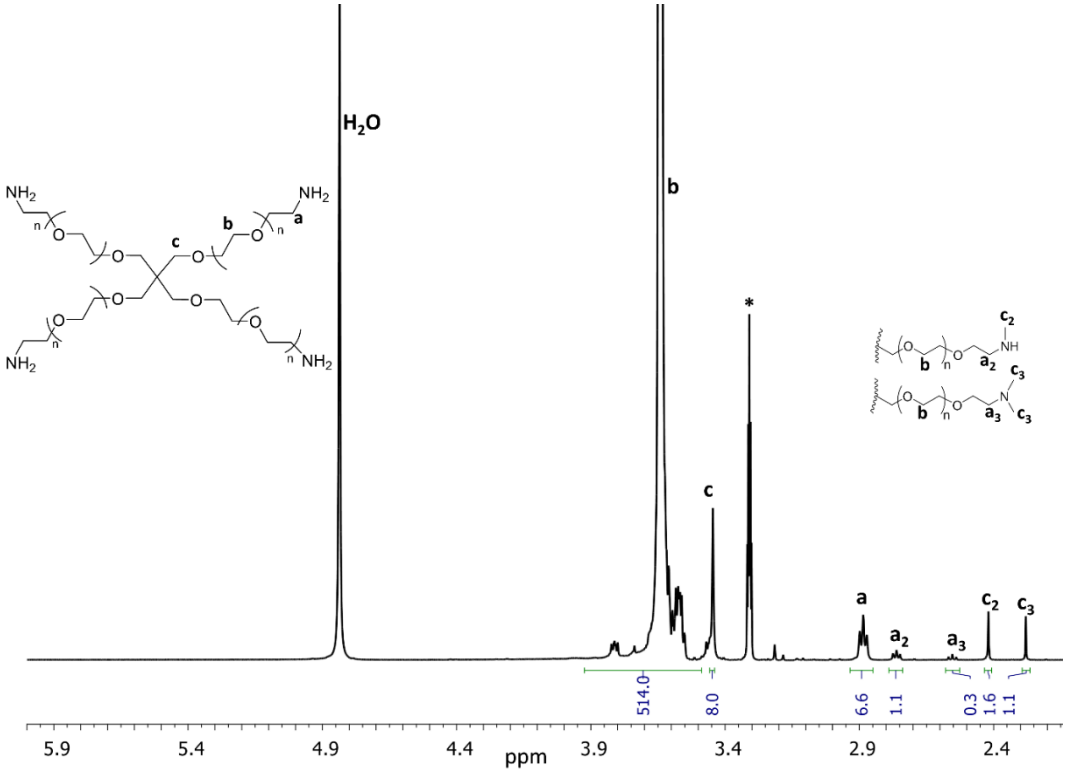


Figure S5. ^{1}H NMR (400 MHz, CD_3OD) spectrum of $[PEG_{\sim 32}\text{-}NH_2]_4$, 4-arm $PEG_{\sim 128}\text{-}NH_2$. Minor amounts of *N*-methyl amine arms are formed, as evidenced by additional triplets from CH_2 protons α to nitrogen (a_2 and a_3 peaks) and the corresponding methyl singlets (c_2 and c_3 peaks). *Residual NMR solvent peak.

3.2.2. 10 kDa 4-Arm PEG-NH_2 ($[PEG_{\sim 55}\text{-}NH_2]_4$) from 4-Arm PEG-OH 10 kDa

Step i: 4-Arm PEG-OH (10 kDa, 3.0 g, 3.0×10^{-4} mol) and TEA (365 mg, 505 µL, 3.6×10^{-3} mol mol) were dissolved in dry DCM (20 mL) under nitrogen. The solution was cooled in an ice bath and then MesCl (344 mg, 232 µL, 3.0×10^{-3} mol) was added dropwise. After the addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was then added to the residue and the white precipitate (residual salt) was removed by filtration. The toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was dropped into excess cold diethyl ether to precipitate the product, **$[PEG_{\sim 50}$-Mesylate$]_4$**, **4-arm $PEG_{\sim 200}$-Mesylate**, as an off-white powder (2.9 g, yield: ~95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.07 (s, 4 × CH_3–SO_3–, 12H), 3.63 (m, –CH_2CH_2–O– of PEG, 200 EG; 4 × 50 EG), 4.35 (t, J = 4.5 Hz, 4 × CH_3SO_3–CH_2–, 8H).

Step ii: The above product **4-arm PEG$_{\sim 200}$-Mesylate** (2.9 g, 3.3×10^{-4} mol) was dissolved in DMF (12 mL) followed by the addition of sodium azide (0.22 g, 3.3 mmol), and the solution was then heated to 75 °C for 16 h. The majority of the DMF was evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled, and filtered, and then the concentrated filtrate was precipitated into cold diethyl ether. The product, **[PEG$_{\sim 50}$-N$_3$]$_4$**, **4-arm PEG$_{\sim 200}$-Azide**, was isolated as an off-white powder (2.3 g, yield: ~80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.37 (t, J = 4.9 Hz, 4 × N_3−CH_2−, 8H), 3.63 (m, –CH_2CH_2–O– of PEG, 200 EG, 4 × 50 EG).

Step iii: The above product **4-arm PEG$_{\sim 200}$-Azide** (2.0 g, 2.4×10^{-4} mol) was dissolved in MeOH (20 mL) followed by the addition of PPh_3 (0.49 g, 1.9×10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **[PEG$_{\sim 55}$-NH$_2$]$_4$**, **4-arm PEG$_{\sim 220}$-NH$_2$**, as an off-white powder (1.8 g, yield: ~90%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.85 (t, J = 5.3 Hz, 4×NH_2−CH_2−, 8H), 3.45 (m, –CH_2CH_2–O– of PEG, 220 EG, 4 × 55 EG). Refer to Figure S6 for peak assignments.

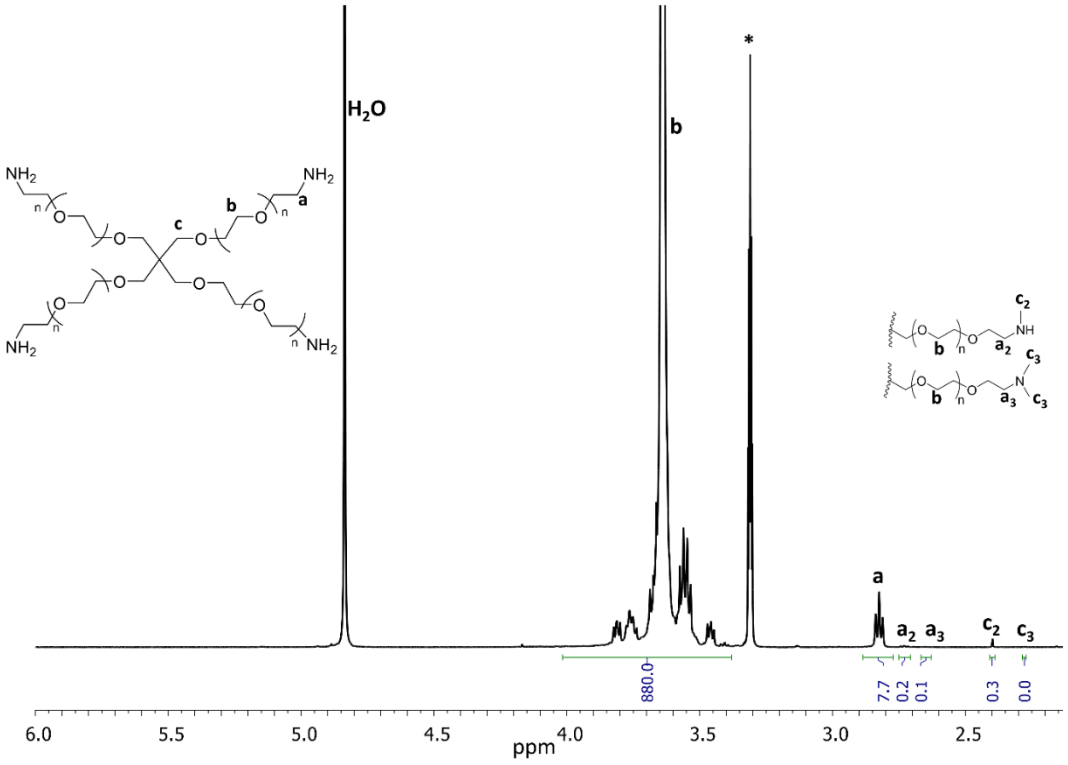
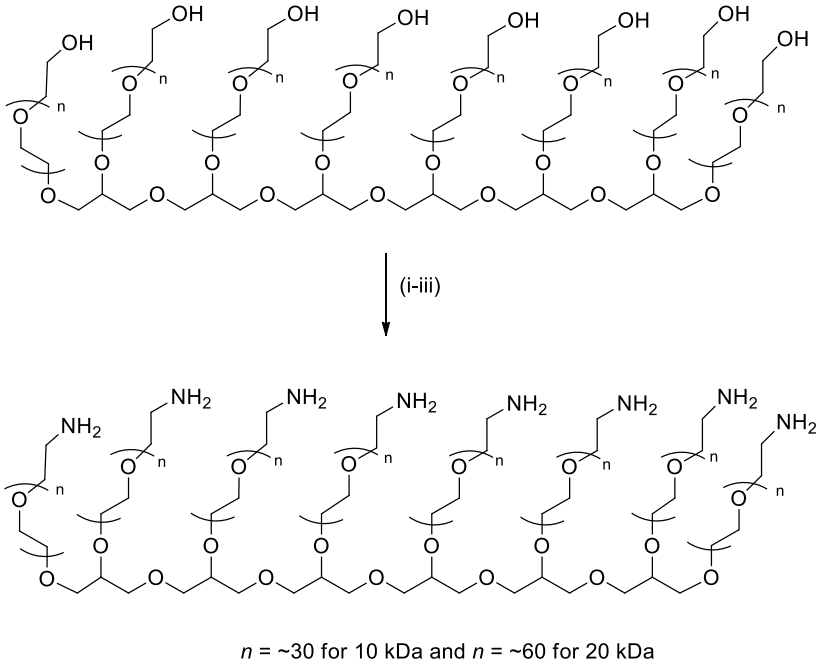


Figure S6. ^{1}H NMR (400 MHz, CD_3OD) spectrum of $[PEG_{\sim 55}\text{-}NH_2]_4$, 4-arm $PEG_{\sim 220}\text{-}NH_2$. Note, CH_2 protons of pentaerythritol core are not evident. Very minor amounts of *N*-methyl amine arms are formed, as evidenced by additional triplets from CH_2 protons α to nitrogen (a_2 and a_3 peaks) and the corresponding methyl singlets (c_2 and c_3 peaks). *Residual NMR solvent peak.

3.3. 8-Arm PEG-NH_2



3.3.1. 10 kDa 8-Arm PEG-NH_2 ($[PEG_{\sim 32}\text{-}NH_2]_8$) from 8-Arm PEG-OH 10 kDa

Step i: 8-Arm PEG-OH (10 kDa, 3.0 g, 3×10^{-4} mol) and TEA (730 mg, 1.0 mL, 7.2×10^{-3} mol mol) were dissolved in dry DCM (20 mL) under nitrogen. The solution was cooled in an ice bath and then MesCl (687 mg, 464 µL, 6.0×10^{-3} mol) was added dropwise. After the addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was then added to the residue and the white precipitate (residual salt) was removed by filtration. Toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was dropped into excess cold diethyl ether to precipitate the product, **$[PEG_{\sim 32}$-Mesylate$]_8$**, **8-arm PEG$_{\sim 256}$-Mesylate**, as an off-white powder (2.8 g, yield: ~93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.06 (s, 8 × C*H*$_3$−SO$_3$−, 24H), 3.63 (m, –CH_2CH_2–O– of PEG, 256 EG; 8 × 32 EG), 4.35 (t, *J* = 4.5 Hz, 8 × CH_3SO$_3$−C*H*$_2$−, 16H) ppm.

Step ii: The above product **8-arm PEG$_{\sim 256}$-Mesylate** (2.8 g, 2.8×10^{-4} mol) was dissolved in DMF (10 mL) followed by the addition of sodium azide (0.36 g, 5.6 mmol) and the solution was then heated to 75 °C for 16 h. The majority of the DMF was evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled, and filtered, and then the concentrated filtrate was precipitated into cold diethyl ether. The product, **$[PEG_{\sim 30}\text{-}N_3]_8$**, **8-arm PEG$_{\sim 240}$-Azide**, was isolated as an off-white powder (2.1 g, yield: ~75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.36 (t, *J* = 4.9 Hz, 8 × N$_3$−C*H*$_2$−, 16H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 240 EG, 8 × 30 EG).

Step iii: The above product **8-arm PEG$_{\sim 240}$-Azide** (2.1 g, 2.1×10^{-4} mol) was dissolved in MeOH (20 mL) followed by the addition of PPh_3 (780 mg, 3.4×10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **$[PEG_{\sim 32}\text{-}NH_2]_8$**, **8-arm PEG$_{\sim 258}$-$NH_2$**, as an off-white powder (1.9 g, yield: ~90 %). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.89 (t, *J* = 5.3 Hz, 8

× NH_2−CH_2−, 16H), 3.45 (m, –CH_2CH_2–O– of PEG, 258 EG, 8 × 32 EG). Refer to Figure S7 for peak assignments.

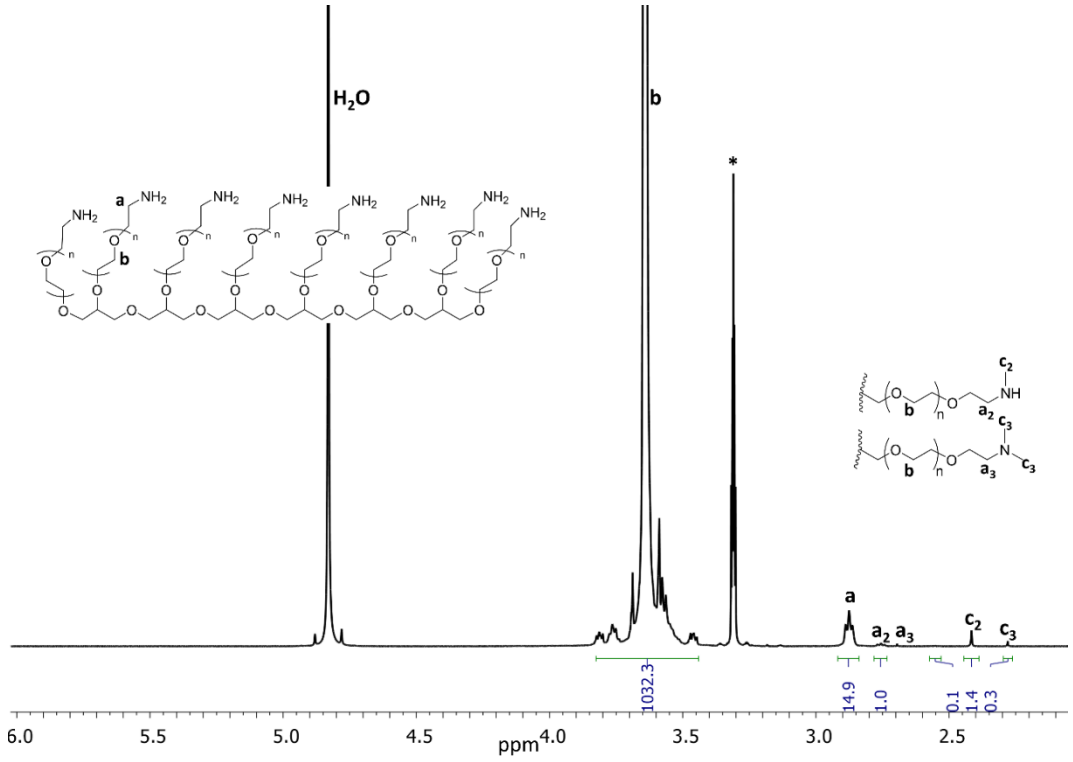


Figure S7. ^{1}H NMR (400 MHz, CD_3OD) spectrum of $[PEG_{\sim 32}\text{-}NH_2]_8$, 8-arm $PEG_{\sim 258}\text{-}NH_2$ showing peak assignments. Very minor amounts of *N*-methyl amine arms formed, evidenced by extra triplets from CH_2 protons α to nitrogen (a_2 and a_3 peaks) and the corresponding methyl singlets (c_2 and c_3 peaks). *Residual NMR solvent peak.

3.3.2. 20 kDa 8-Arm PEG-NH_2 ($[PEG_{\sim 64}\text{-}NH_2]_8$) from 8-Arm PEG-OH 20 kDa

Step i: 8-Arm PEG-OH (20 kDa, 2.3 g, 1.15×10^{-4} mol) and TEA (279 mg, 384 µL, 2.8×10^{-3} mol) were dissolved in dry DCM (15 mL) under nitrogen. The solution was cooled in an ice bath and then MesCl (263 mg, 178 µL, 2.3×10^{-3} mol) was added dropwise. After addition was complete, the solution was stirred at ambient temperature under nitrogen for 16 h. The solvent was then removed under reduced pressure. Toluene was then added to the residue and the white precipitate (residual salt) was removed by filtration. Toluene was then removed under reduced pressure from the filtrate and the concentrated filtrate was dropped into excess cold diethyl ether to precipitate the product, **$[PEG_{\sim 60}\text{-Mesylate}]_8$**, **8-arm $PEG_{\sim 480}$-Mesylate**, as an off-white powder (~2.3 g, yield: quantitative). ^{1}H NMR

(400 MHz, $CDCl_3$) δ ppm: 3.06 (s, 8 × C*H*$_3$−SO_3−, 24H), 3.63 (m, –CH_2CH_2–O– of, 480 EG; 8 × 60 EG), 4.36 (m, 8 × CH_3SO_3−C*H*$_2$−, 16H).

Step ii: The above product **8-arm PEG$_{\sim 480}$-Mesylate** (1.7 g, 0.85 × 10^{-4} mol) was dissolved in DMF (8 mL) followed by the addition of sodium azide (0.11 g, 1.7 × 10^{-3} mol) and the solution was then heated to 75 °C for 16 h. The majority of the DMF was evaporated using high vacuum and the residue was further dried by passing a stream of air over the residue overnight. The solid was then taken up in CH_2CI_2, cooled, and filtered, and then the concentrated filtrate was precipitated into cold diethyl ether. The product, **[PEG$_{\sim 60}$-N$_3$]$_8$**, **8-arm PEG$_{\sim 480}$-Azide**, was isolated as an off-white powder (1.3 g, yield: ~76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.38 (t, *J* = 4.9 Hz, 8 × N_3−C*H*$_2$−, 16H), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 480 EG, 8 × 60 EG).

Step iii: The above product **8-arm PEG$_{\sim 480}$-Azide** (1.2 g, 6.2 × 10^{-5} mol) was dissolved in MeOH (15 mL) followed by the addition of PPh_3 (323 mg, 1.2 × 10^{-3} mol). The solution was heated under reflux at 80 °C for 16 h. MeOH was then removed in vacuo and the residue was dissolved in CH_2Cl_2 and precipitated into diethyl ether twice to give the product, **[PEG$_{\sim 64}$-NH$_2$]$_8$**, **8-arm PEG$_{\sim 512}$-NH$_2$**, as an off-white powder (1.1 g, yield: ~92 %). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 2.89 (t, *J* = 5.3 Hz, 8 × NH_2−C*H*$_2$−, 16H), 3.45 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 512 EG, 8 × 58 EG) ppm. Refer to Figure S8 for peak assignments.

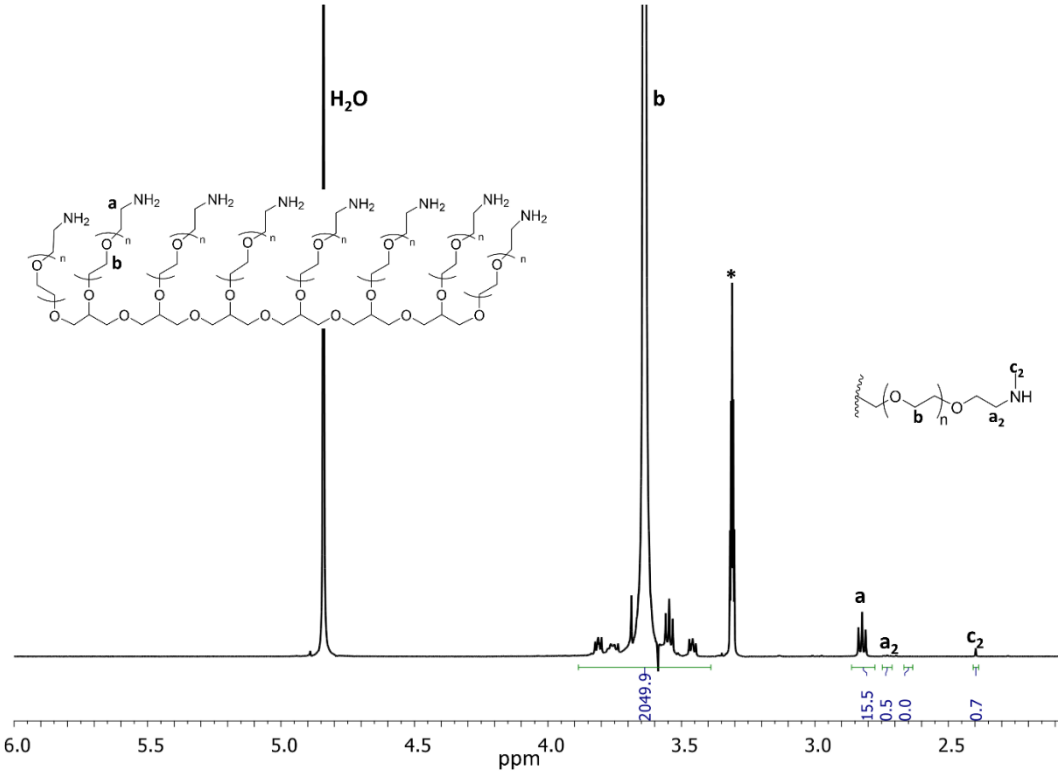
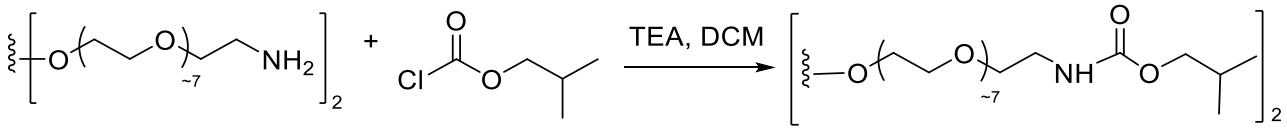


Figure S8. ^{1}H NMR (400 MHz, CD_3OD) spectrum of $[PEG_{\sim 64}\text{-}NH_2]_8$, 8-arm $PEG_{\sim 512}\text{-}NH_2$. Very minor amounts of *N*-methyl amine arms formed, as evidenced by additional triplets from CH_2 protons α to nitrogen (a_2) and the corresponding methyl singlets (c_2 peaks). *Residual NMR solvent peak.

4. ^{1}H NMR Identification of Side Product [iBoc-PEG$_7$ carbamate]$_2$ (see Scheme S2)



2-Arm PEG-NH$_2$, ([PEG$_{\sim 7}$-NH$_2$]$_2$) (165 mg, 2.6×10^{-4} mol) was added to an oven-dried vial and to this was added dry DCM (1mL) followed by TEA (60 mg, 82 µL, 5.9×10^{-4} mol). The solution was capped with a rubber septum and kept under nitrogen while cooling using an ice bath. Isobutyl chloroformate (77 mg, 73 µL, 5.6×10^{-4} mol) was added dropwise after which the solution was left to stir at ambient temperature for 2 h. Hexane (20 mL) was then added to the reaction mixture and the solids were then filtered out. The filtrate was evaporated and the resulting mixture was purified by silica gel flash chromatography EtOAc/hexane (EtOAc/DCM (1:1 v/v) → 5% (v/v) MeOH in DCM) to afford the product **[iBoc-PEG$_{\sim 7}$ carbamate]$_2$**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.82 (2H, d, *J* =

6.5 Hz, CH_2, isobutyl), 3.63 (m, –C*H*$_2$C*H*$_2$–O– of PEG, 14 EG, 2 × 7 EG), 3.33–3.37 (4H, m, –C*H*$_2$–NHCO–), 1.84–1.95 (2H, m, 2 × –CH, isobutyl), 0.90 (d, *J* = 6.7 Hz, 4 × –CH_3, isobutyl, 12H). Refer to Figure S9 for peak assignments.

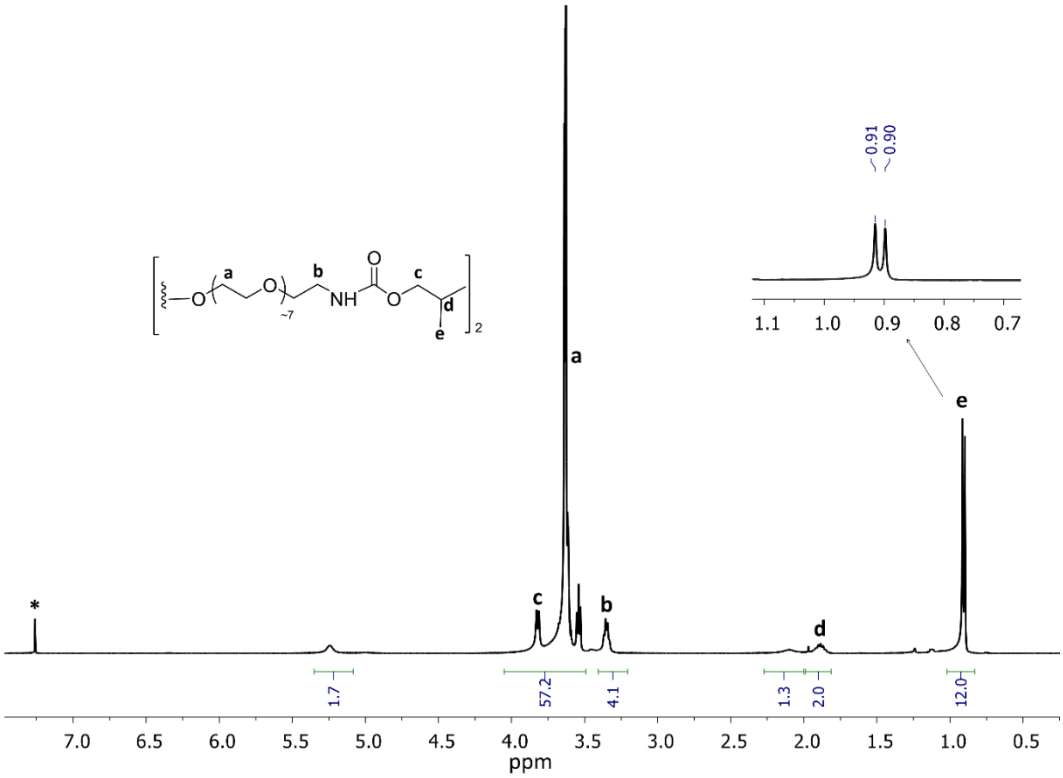


Figure S9. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [iBoc-PEG$_{\sim 7}$ carbamate]$_2$. *Residual NMR solvent peak.

5. Synthesis of Multi-Arm PEG-caffeamide Building Blocks ([PEG$_n$-caffeamide]$_{2/4/8}$)

5.1. 2.5 kDa 2-Arm PEG-CAF ([PEG$_{\sim 37}$-caffeamide]$_2$) from [PEG$_{28}$-NH$_2$]$_2$

2-Arm PEG-NH$_2$, [PEG$_{\sim 28}$-NH$_2$]$_2$ prepared in-house as described above (375 mg, 1.56×10^{-4} mol) was weighed into a 4 mL vial. Dry DCM (1.5 mL) was then added via a rubber septum and the solution stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (300 mg, 6.24×10^{-4} mol) followed by dry DCM (3.0 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 28}$-NH$_2$]$_2$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min.

The solution was then concentrated to half its original volume and then added dropwise into excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 28}$-iBoc-caffeamide]$_2$**, **2-arm PEG-iBocCAF**, as a sticky clear residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 96%). Refer to Figure S10 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 28}$ carbamate]** arms, which form as a by-product of the reaction, as a result of the reaction between the NH_2 groups of PEG and the carbonate groups (see Scheme S2).

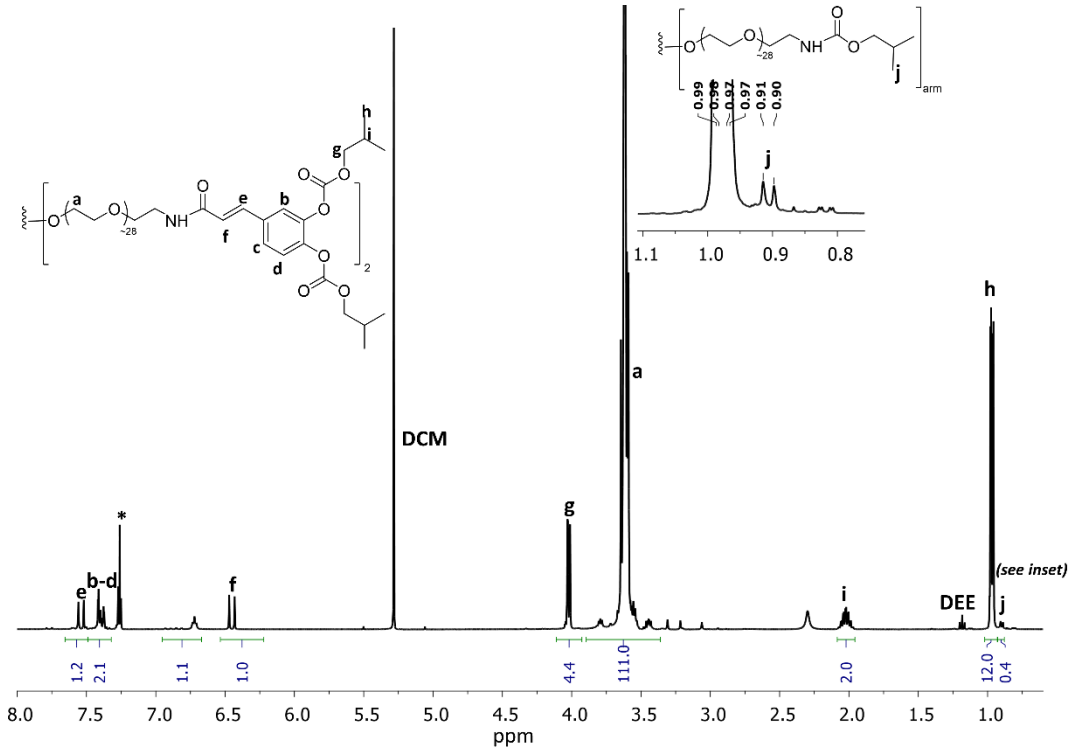


Figure S10. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 28}$-iBoc-caffeamide]$_2$, 2-arm PEG-iBocCAF prepared from [PEG$_{\sim 28}$-NH_2]$_2$ (2.5 kDa). Peaks **j** shown in inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{28}$-iBoc-caffeamide]$_2$ from the previous step (400 mg, 12.8×10^{-5} mol) was dissolved in dry DCM (3.2 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (840 µL of isopropylamine in 1.80 mL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to isopropylamine/DCM solution under stirring. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of

isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product **[PEG$_{\sim 37}$-caffeamide]$_2$** as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~80%). Refer to Figure S11 for peak assignments.

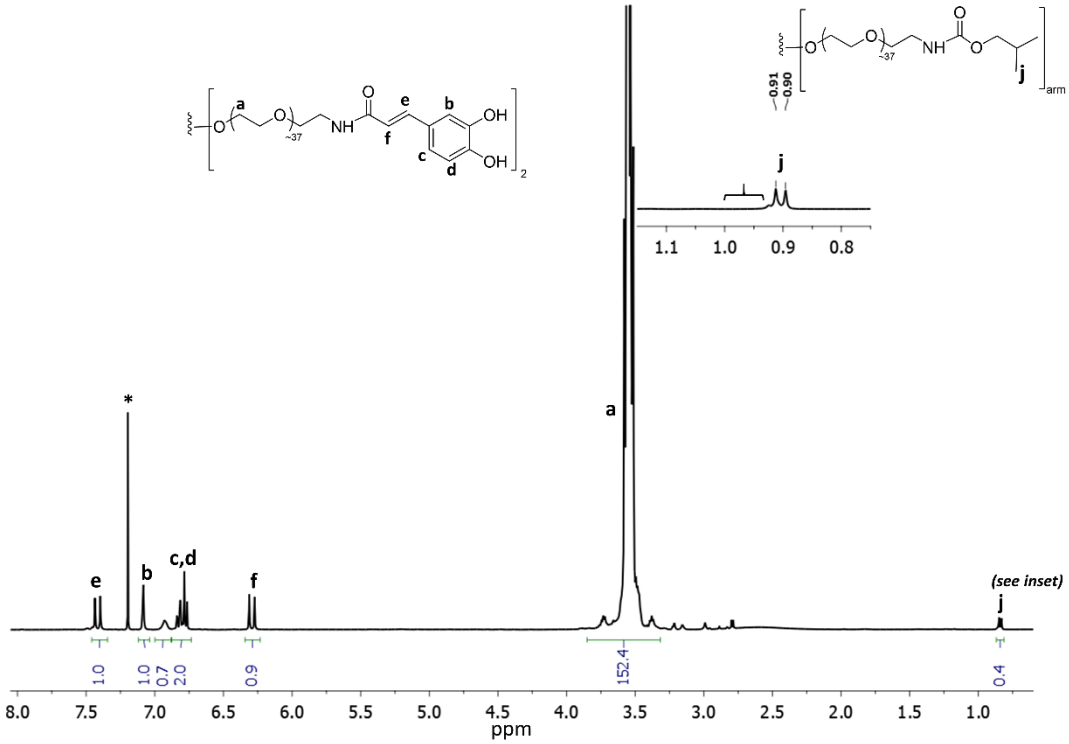


Figure S11. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 37}$-caffeamide]$_2$, 2-arm PEG-CAF prepared from [PEG$_{\sim 28}$-iBoc-caffeamide]$_2$. The inset shows the absence of isobutoxy carbonyl (4× –CH_3) proton signals originally from the protected end groups. Peaks **j** shown in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.2. 2 kDa 2-Arm PEG-CAF ([PEG$_{\sim 36}$-caffeamide]$_2$) from [PEG$_{30}$-NH$_2$]$_2$

2-Arm PEG-NH$_2$, [PEG$_{\sim 30}$-NH$_2$]$_2$ (Sigma-Aldrich, 2.6 kDa, 271 mg, 1.04×10^{-4} mol) was weighed into a 4 mL vial. Dry DCM (2.5 mL) was then added via a rubber septum and the solution was stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (200 mg, 4.16×10^{-4} mol) followed by dry DCM (2.0 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 30}$-NH$_2$]$_2$** was then taken up into a syringe and slowly

added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 32}$-iBoc-caffeamide]$_2$**, **2-arm PEG-iBocCAF**, as a sticky clear residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 95%). Refer to Figure S12 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 32}$ carbamate]** arms which form as a by-product, as a result of the reaction between the NH_2 groups of PEG and the carbonate groups (see Scheme S2).

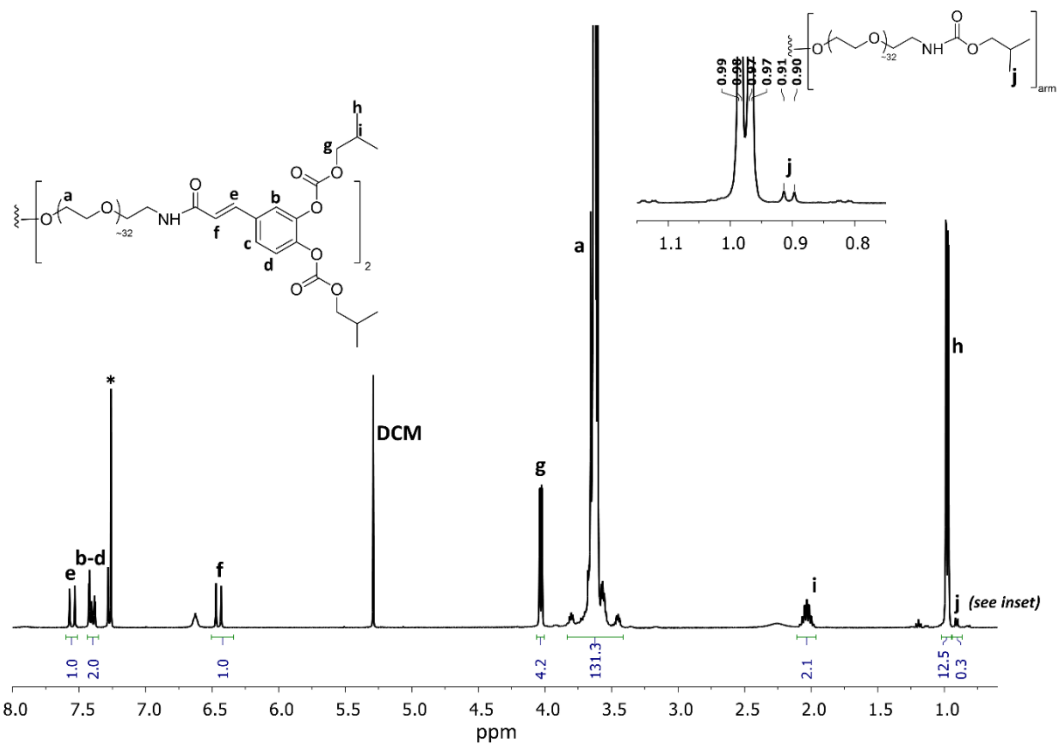


Figure S12. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 32}$-iBoc-caffeamide]$_2$, 2-arm PEG-iBocCAF prepared from [PEG$_{\sim 30}$-NH_2]$_2$ (2.6 kDa). Peaks **j** shown in inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 32}$-iBoc-caffeamide]$_2$ from the previous step (250 mg, 7.1 × 10^{-5} mol) was dissolved in dry DCM (1.30 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (500 µL of isopropylamine in 1.06 mL DCM). The PEG-iBocCAF solution was then added

dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 36}$-caffeamide]$_2$**, as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~90%). Refer to Figure S13 for peak assignments. The 2-arm PEG-CAF was used for capsule fabrication and further referred to as **2.5 kDa 2-arm PEG-CAF** in capsule analysis.

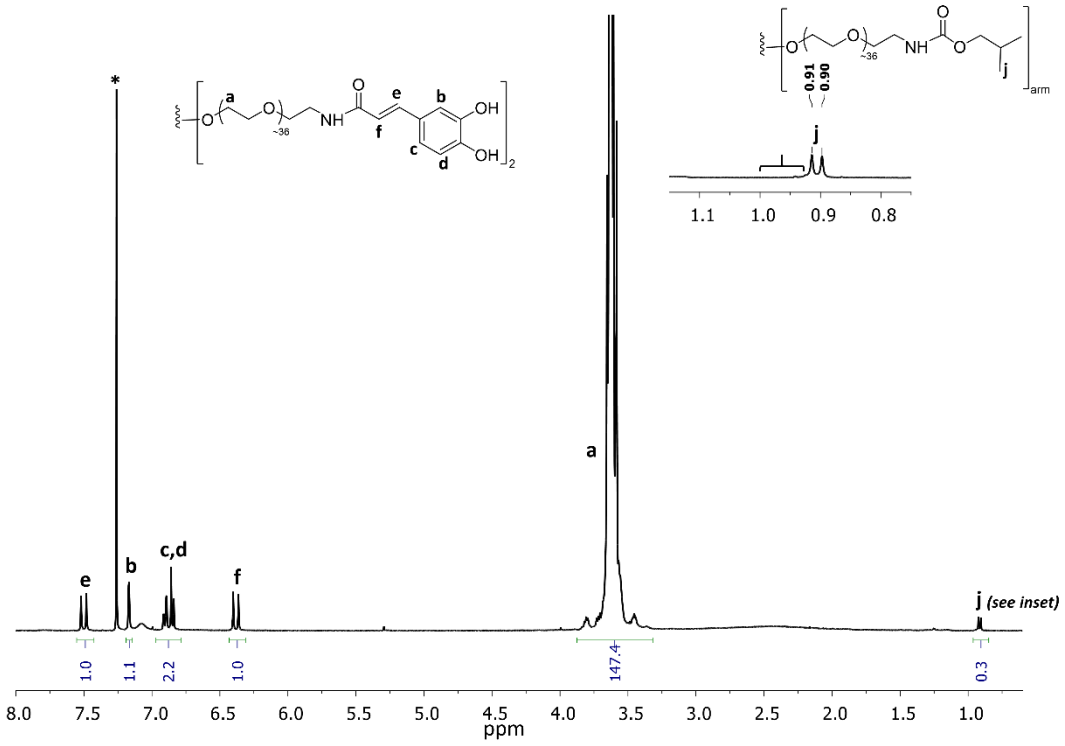


Figure S13. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 36}$-caffeamide]$_2$, 2-arm PEG-CAF prepared from [PEG$_{\sim 32}$-iBoc-caffeamide]$_2$. Inset shows the disappearance of isobutoxy carbonyl (4× -CH_3) proton signals, originally from the protected end groups. Peaks **j** shown in inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.3. 2.5 kDa 2-Arm PEG-CAF ([PEG$_{\sim 36}$-caffeamide]$_2$) Using 1-Step Procedure (Conjugation Followed Directly by Deprotection)

Although a two-step strategy for conjugation and deprotection was implemented which involves two separate isolation steps, similar results could be achieved by bypassing the intermediate isolation step for the protected [PEG$_n$-iBoc-caffeamide]$_{2/4/8}$ (as described below). However, as observed from Figure S14, a minor amount of the protected arm was found to remain after deprotection (<2%). This is likely due to the deprotection solution being too dilute in amine, as the amount of DCM is not adjusted for the deprotection step. Furthermore, it is likely that some of the amine deprotecting agent is sacrificed by reacting with excess iBocCAF which remains in the reaction mixture when the amine is added. It is therefore preferable to carry out the reactions as two separate steps to guarantee complete deprotection.

2-Arm PEG-NH$_2$, [PEG$_{\sim 30}$-NH$_2$]$_2$ (Sigma-Aldrich, 2.6 kDa, 250 mg, 0.96×10^{-4} mol) was weighed into a 4 mL vial. Dry DCM (2.0 mL) was then added via a rubber septum and the solution was stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (190 mg, 4.0×10^{-4} mol) followed by dry DCM (2.3 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 30}$-NH$_2$]$_2$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. An aliquot of isopropyl amine was added directly to the stirring solution (500 µL, 6.1×10^{-3} mol). The final concentration of isopropylamine in the reaction was ~10% v/v or 1.3 M. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product **[PEG$_{\sim 36}$-caffeamide]$_2$** as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~70%). Refer to Figure S14 for peak assignments.

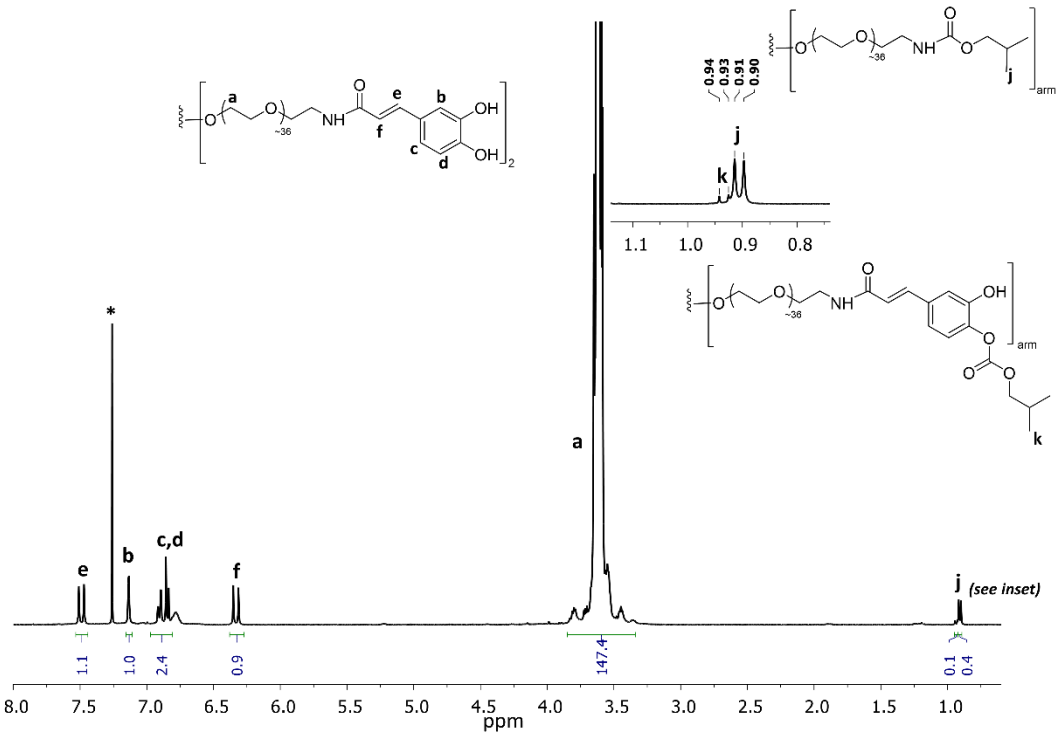


Figure S14. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 36}$-caffeamide]$_2$, 2-arm PEG-CAF prepared in one step from [PEG$_{\sim 30}$-NH$_2$]$_2$ (2.6 kDa). Peaks **j** shown in inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. Peaks **k** are from isobutyl CH_3 protons of [PEG$_{\sim 36}$-iBoc-caffeamide] arms that are not fully deprotected. *Residual NMR solvent peak.

5.4. 5 kDa 2-Arm PEG-CAF ([PEG$_{\sim 65}$-caffeamide]$_2$) from [PEG$_{\sim 59}$-NH$_2$]$_2$

2-Arm PEG-NH$_2$, [PEG$_{\sim 59}$-NH$_2$]$_2$ prepared in-house as described above (470 mg, 9.03×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (2.0 mL) was then added via a rubber septum and the solution stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (140 mg, 2.91×10^{-4} mol) followed by dry DCM (1.4 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 59}$-NH$_2$]$_2$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 60}$-iBoc-caffeamide]$_2$**, **[PEG$_{\sim 60}$-iBoc-CAF]$_2$**, as a sticky residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 94%). Refer to Figure S15 for peak assignments. The ratio of integral values for peaks

h and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 60}$ carbamate]** arms which form as a by-product, as a result of the reaction between the NH_2 groups of PEG and the carbonate groups (see Scheme S2).

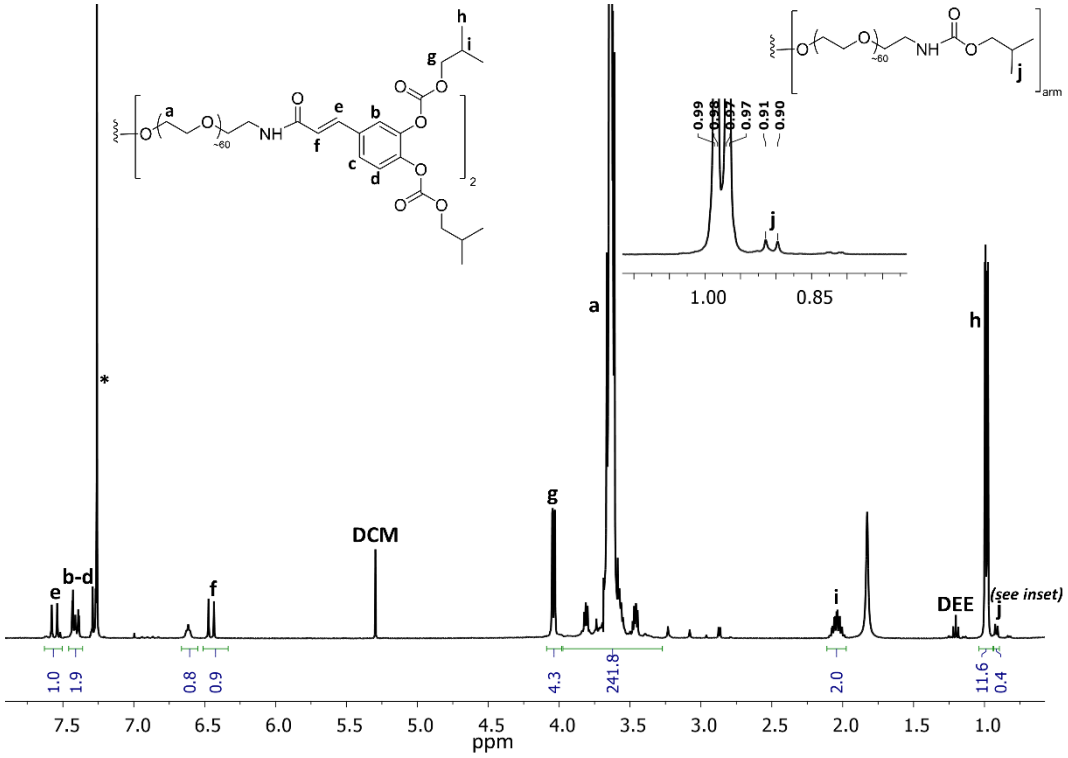


Figure S15. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 60}$-iBoc-caffeamide]$_2$, 2-arm PEG-iBocCAF prepared from [PEG$_{\sim 59}$-NH_2]$_2$ (~5 kDa). Peaks **j** shown in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 60}$-iBoc-caffeamide]$_2$ (**2-arm PEG$_{\sim 120}$-iBocCAF**) from the previous step (535 mg, 7.98 × 10^{-5} mol) was dissolved in dry DCM (1.2 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (520 µL of isopropylamine in 1.2 mL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise into excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 65}$-caffeamide]$_2$**, as a sticky tan/brown residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against

acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream/tan) (yield: ~90%). Refer to Figure S16 for peak assignments.

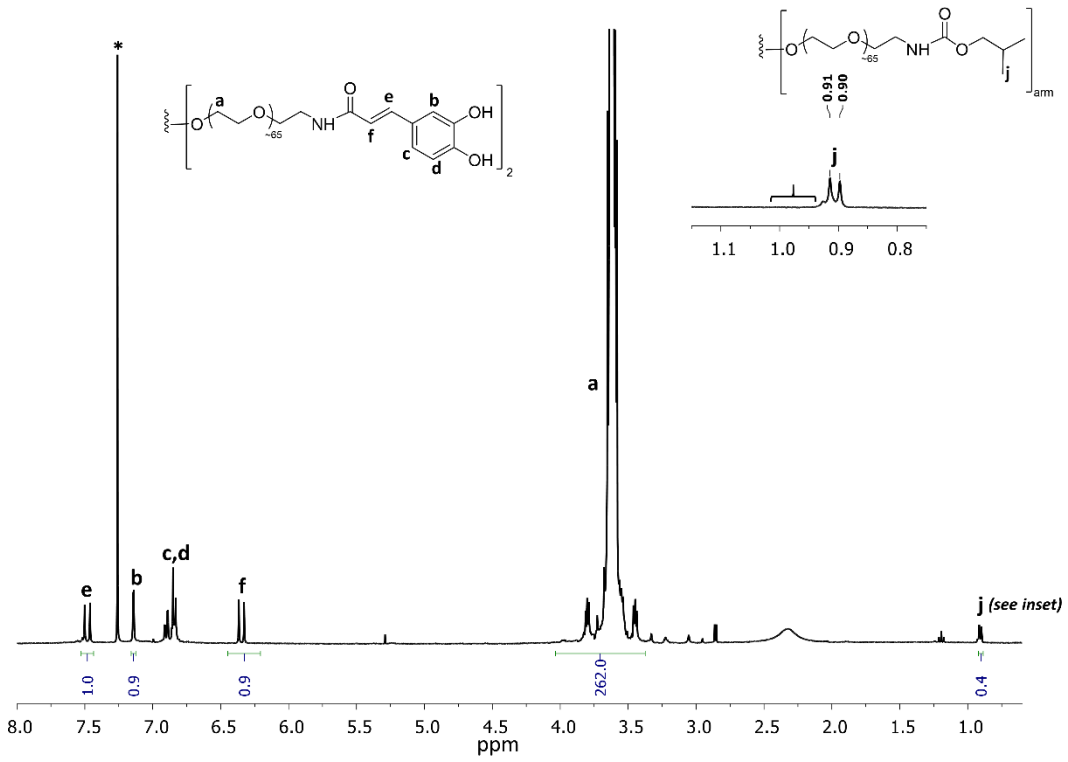


Figure S16. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of $[PEG_{\sim 65}\text{-caffeamide}]_2$, 2-arm PEG-CAF prepared from $[PEG_{\sim 60}\text{-iBoc-caffeamide}]_2$. The inset shows the absence of isobutoxy carbonyl (4× $-CH_3$) proton signals originally from the protected end groups. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.5. 5 kDa 2-Arm PEG-CAF ($[PEG_{\sim 90}\text{-caffeamide}]_2$) from $[PEG_{\sim 69}\text{-}NH_2]_2$

2-Arm PEG-NH_2, $[PEG_{\sim 69}\text{-}NH_2]_2$ (Creative PEGWorks, 419 mg, 8.39×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (2.0 mL) was then added via a rubber septum and the solution stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (130 mg, 2.70×10^{-4} mol) followed by dry DCM (1.4 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **$[PEG_{\sim 69}\text{-}NH_2]_2$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold

diethyl ether to precipitate the product, **[PEG$_{\sim 85}$-iBoc-caffeamide]$_2$**, **2-arm PEG-iBocCAF**, as a sticky clear residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 93%). Refer to Figure S17 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 85}$-carbamate]** arms which form as a by-product, as a result of the reaction of the NH_2 groups of PEG with carbonate groups (Scheme S2).

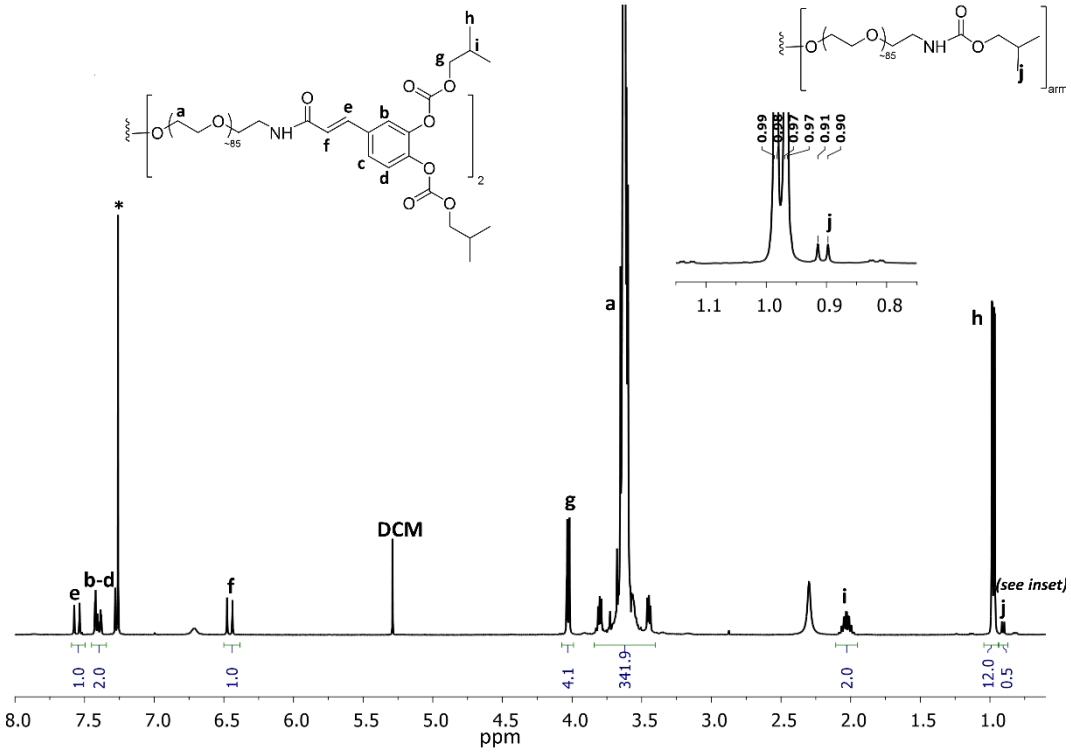


Figure S17. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 85}$-iBoc-caffeamide]$_2$, 2-arm PEG-iBocCAF prepared from [PEG$_{\sim 69}$-NH_2]$_2$ (~5 kDa). Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 85}$-iBoc-caffeamide]$_2$ from the previous step (376 mg, 4.6×10^{-5} mol) was dissolved in dry DCM (0.85 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (300 µL of isopropylamine in 0.64 mL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature

under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product **[PEG$_{\sim 90}$-caffeamide]$_2$** as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~90%). Refer to Figure S18 for peak assignments. The 2-arm PEG-CAF was used for capsule fabrication and further referred to as **5 kDa 2-arm PEG-CAF** in capsule analysis.

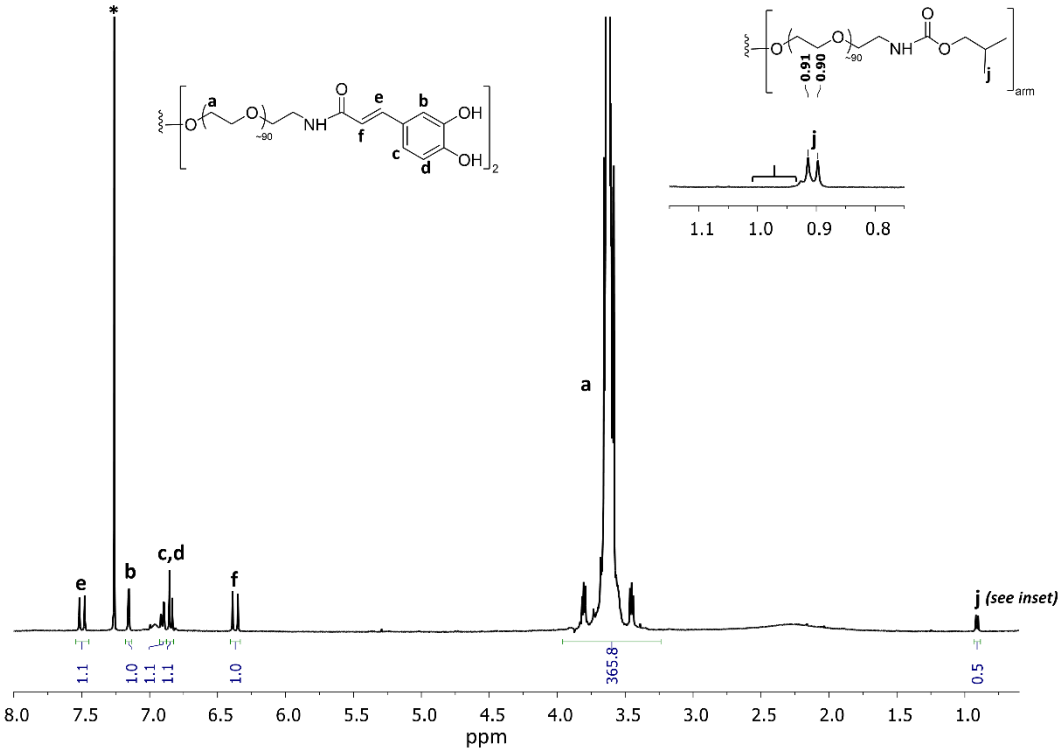


Figure S18. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 90}$-caffeamide]$_2$, 2-arm PEG-CAF prepared from [PEG$_{\sim 85}$-iBoc-caffeamide]$_2$. Inset shows the absence of isobutoxy carbonyl (4× –CH_3) proton signals originally from the protected end groups. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.6. 5 kDa 4-Arm PEG-CAF ([PEG$_{\sim 35}$-caffeamide]$_4$) from [PEG$_{\sim 32}$-NH$_2$]$_4$

4-Arm PEG-NH$_2$, [PEG$_{\sim 32}$-NH$_2$]$_4$, prepared in-house as described above (377 mg, 6.73×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (1.3 mL) was then added via a rubber septum and the solution stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (258 mg, 5.37×10^{-4} mol) followed by dry DCM (2.5 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 32}$-NH$_2$]$_4$** was then taken up into a syringe and slowly

added dropwise to the solution of iBocCAF. After the addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 35}$-iBoc-caffeamide]$_4$**, **[PEG$_{\sim 35}$-iBocCAF]$_4$**, as a sticky residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 95%). Refer to Figure S19 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peak **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 35}$-carbamate]** arms which form as a by-product, as a result of the reaction of the NH_2 groups of PEG with carbonate groups (Scheme S2).

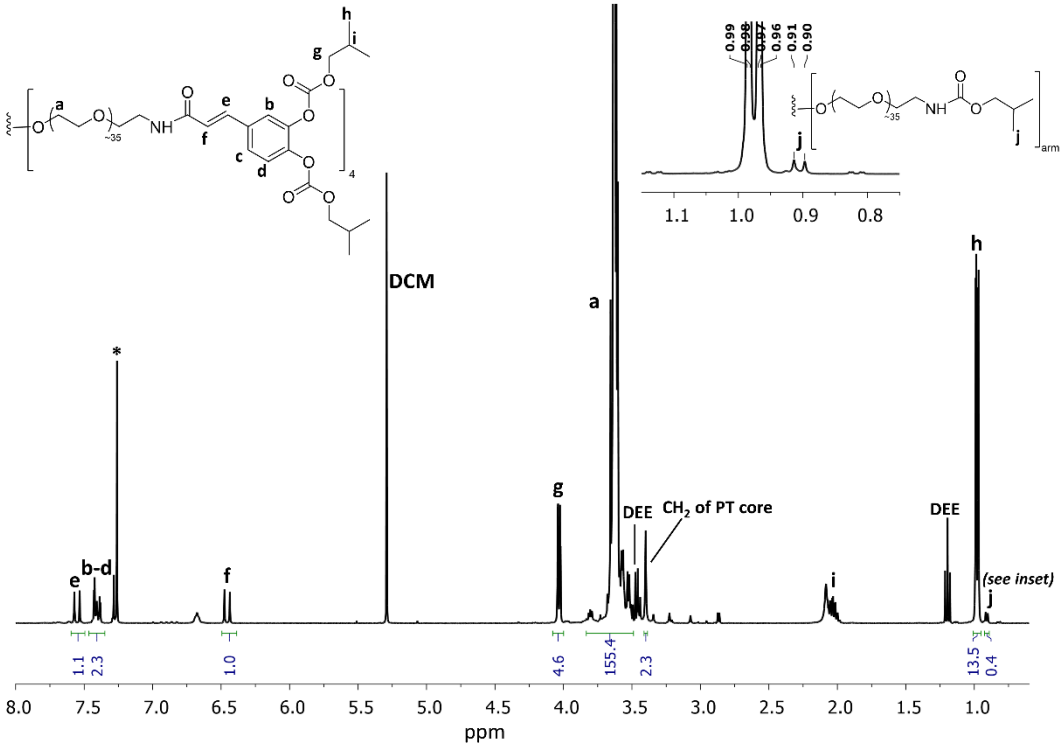


Figure S19. ^{1}H NMR (400 MHz, $CDCl_3$) of [PEG$_{\sim 35}$-iBoc-caffeamide]$_4$, 4-arm PEG-iBocCAF prepared from [PEG$_{\sim 32}$-NH_2]$_4$ (~5.6 kDa). Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate]$_2$ arms which are a by-product of the reaction. Pentaerythritol (PT) core which is not shown in the structure features CH_2 peaks in the ^{1}H NMR spectrum (as indicated). *Residual NMR solvent peak.

[PEG$_{\sim 35}$-iBoc-caffeamide]$_4$ from the previous step (440 mg, 6.0 × 10^{-5} mol) was dissolved in dry DCM (2.2 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (800 µL of isopropylamine in 1.7 mL DCM). The PEG-iBocCAF solution was then added

dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product **[PEG$_{\sim 35}$-caffeamide]$_4$** as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~80%). Refer to Figure S20 for peak assignments. The 4-arm PEG-CAF was used for capsule fabrication and further referred to as **5 kDa 4-arm PEG-CAF** in capsule analysis.

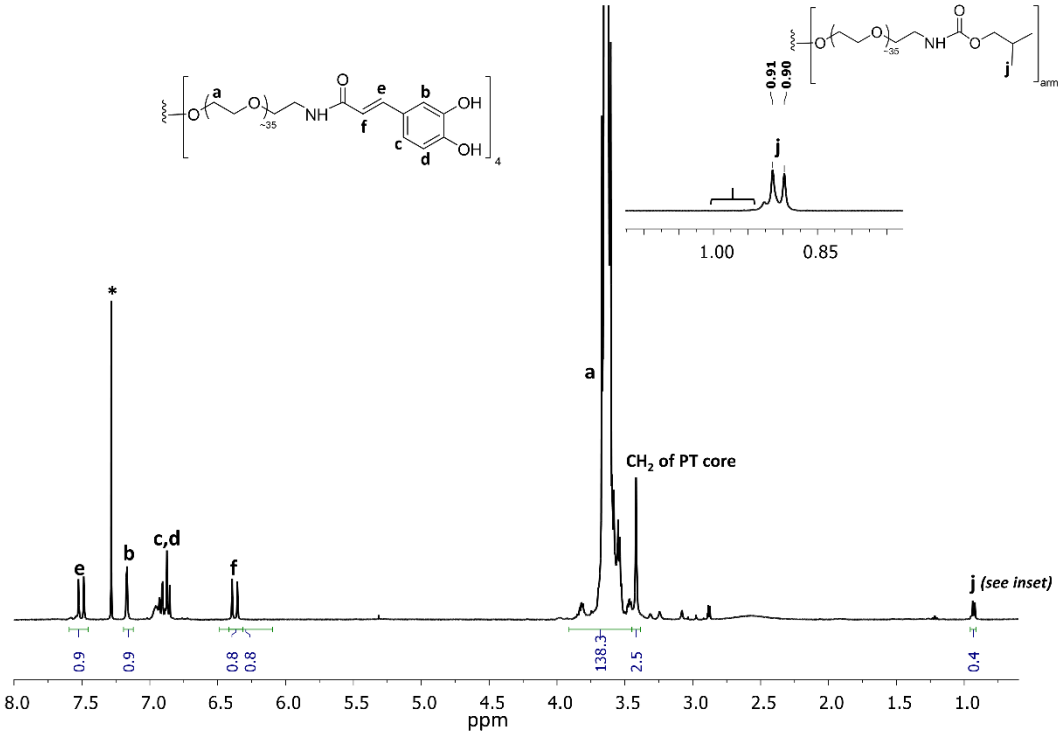


Figure S20. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 35}$-caffeamide]$_4$, 4-arm PEG-CAF prepared from [PEG$_{\sim 35}$-iBoc-caffeamide]$_4$. Inset shows the absence of isobutoxy carbonyl (4× –CH_3) proton signals originally from the protected end groups. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG$_{\sim 35}$ carbamate]$_4$ arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.7. 10 kDa 4-Arm PEG-CAF ([PEG$_{\sim 64}$-caffeamide]$_4$) from [PEG$_{\sim 55}$-NH$_2$]$_4$

4-Arm PEG-NH$_2$, [PEG$_{\sim 55}$-NH$_2$]$_4$ prepared in-house as described above (330 mg, 3.3×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (2.5 mL) was then added via a rubber septum and the solution

stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (127 mg, 2.6×10^{-4} mol) followed by dry DCM (1.2 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 55}$-NH$_2$]$_4$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After the addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 59}$-iBoc-caffeamide]$_4$, [PEG$_{\sim 59}$-iBocCAF]$_4$**, as a sticky residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 95%). Refer to Figure S21 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 59}$-carbamate]** arms which form as a by-product, as a result of the reaction of the NH_2 groups of PEG with carbonate groups (Scheme S2).

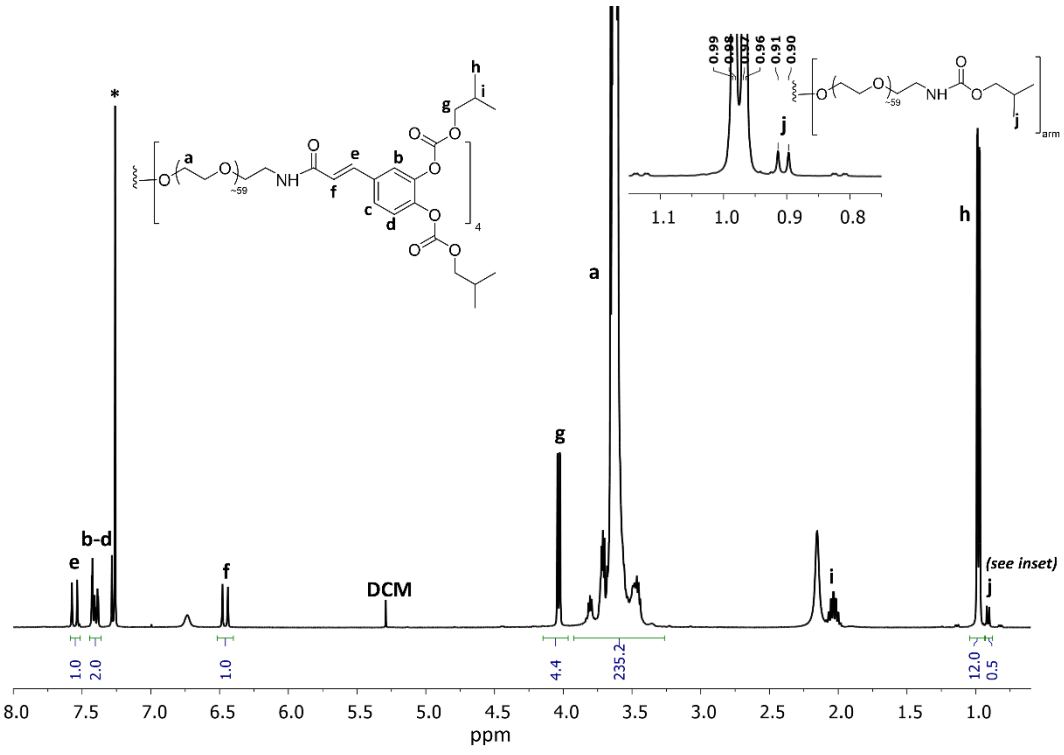


Figure S21. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 59}$-iBoc-caffeamide]$_4$, 4-arm PEG-iBocCAF prepared from [PEG$_{\sim 55}$-NH$_2$]$_4$ (~10 kDa). Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 59}$-iBoc-caffeamide]$_4$ described above (320 mg, 2.7×10^{-5} mol) was dissolved in dry DCM (1.0 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (350 µL of isopropylamine in 740 µL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product **[PEG$_{\sim 64}$-caffeamide]$_4$** as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~75%). Refer to Figure S22 for peak assignments. The 4-arm PEG-CAF was used for capsule fabrication and further referred to as **10 kDa 4-arm PEG-CAF** in capsule analysis.

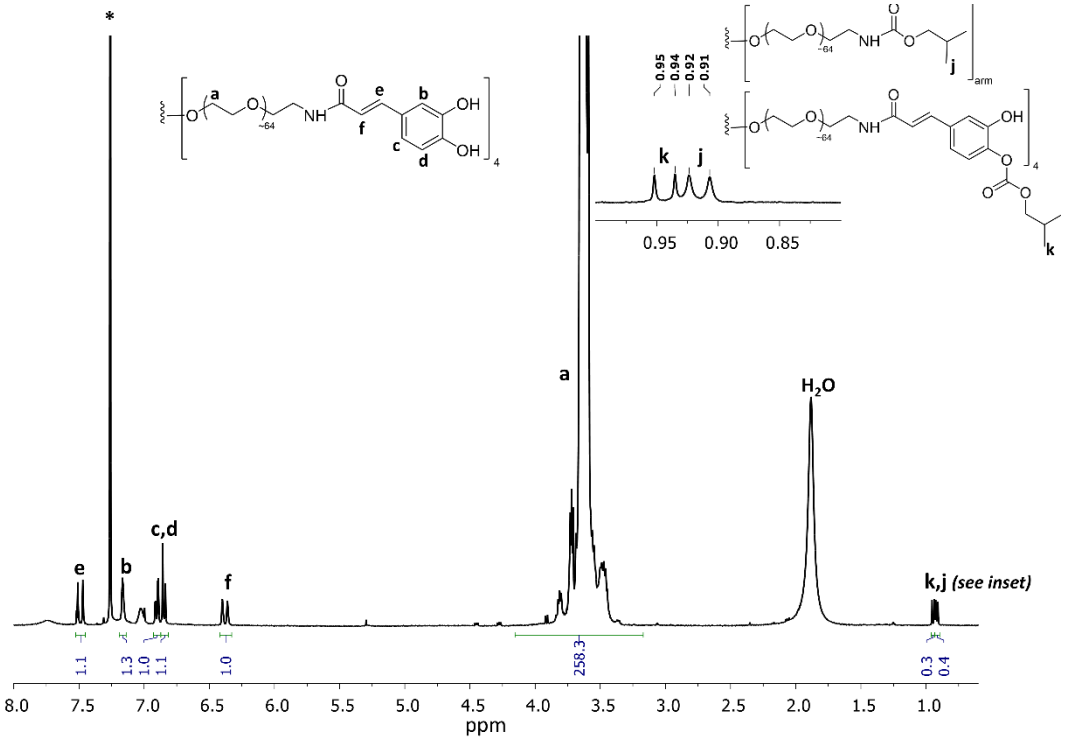


Figure S22. ^{1}H NMR (400 MHz, $CDCl_3$) of [PEG$_{\sim 64}$-caffeamide]$_4$, 4-arm PEG-CAF prepared from [PEG$_{\sim 59}$-iBoc-caffeamide]$_4$. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate]$_4$ arms (by-product) which remain after purification. Peaks **k** are from isobutoxy carbonyl CH_3 protons of [PEG$_{\sim 64}$-iBoc-caffeamide] arms that are not fully deprotected. *Residual NMR solvent peak.

5.8. 10 kDa 8-Arm PEG-CAF ([PEG$_{\sim 35}$-caffeamide]$_8$) from [PEG$_{\sim 32}$-NH$_2$]$_8$

8-Arm PEG-NH$_2$, [PEG$_{\sim 32}$-NH$_2$]$_8$, prepared in-house as described above (276 mg, 2.6×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (1.4 mL) was then added via a rubber septum and the solution was stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (200 mg, 4.2×10^{-4} mol), followed by dry DCM (2.0 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[PEG$_{\sim 32}$-NH$_2$]$_8$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After the addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 33}$-iBoc-caffeamide]$_8$**, **[PEG$_{\sim 33}$-iBoc-CAF]$_8$**, as a sticky residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 93%). Refer to Figure S23 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peaks **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 33}$-carbamate]** arms which form as a by-product as a result of the reaction of the NH_2 groups of PEG with carbonate groups (Scheme S2).

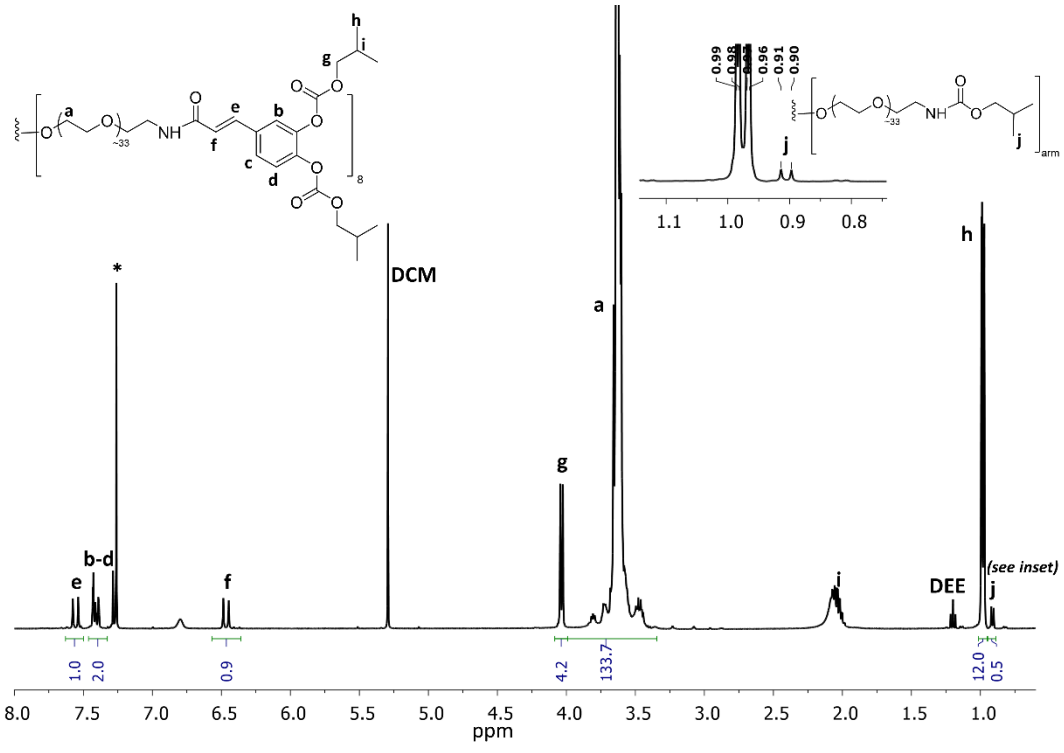


Figure S23. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 33}$-iBoc-caffeamide]$_8$, 8-arm PEG-iBocCAF prepared from [PEG$_{\sim 32}$-NH_2]$_8$ (~10 kDa). Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 33}$-iBoc-caffeamide]$_8$ described above (295 mg, 2.7×10^{-5} mol) was dissolved in dry DCM (1.5 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (533 µL of isopropylamine in 1.13 mL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per iso-butyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 35}$-caffeamide]$_8$**, as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~70%). Refer to Figure S24 for peak assignments. The 8-arm PEG-CAF was used for capsule fabrication and further referred to as **10 kDa 8-arm PEG-CAF** in capsule analysis.

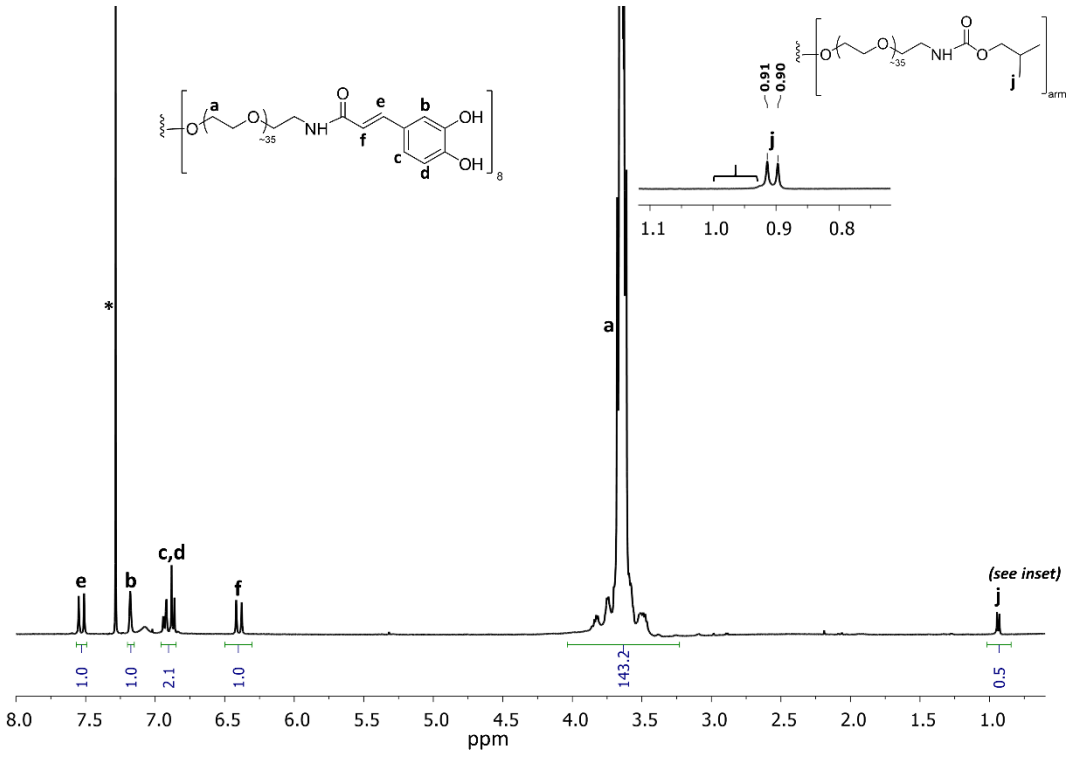


Figure S24. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [$PEG_{\sim 35}$-caffeamide]$_8$, 8-arm PEG-CAF prepared from [$PEG_{\sim 33}$-iBoc-caffeamide]$_8$. Inset shows the absence of isobutoxy carbonyl (4× –CH_3) proton signals originally from the protected end groups. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

5.9. 20 kDa 8-Arm-PEG-CAF ([$PEG_{\sim 64}$-caffeamide]$_8$) from [$PEG_{\sim 64}$-NH_2]$_8$

8-Arm PEG-NH_2, [$PEG_{\sim 64}$-NH_2]$_8$ prepared in-house as described above (389 mg, 1.7×10^{-5} mol) was weighed into a 4 mL vial. Dry DCM (2.0 mL) was then added via a rubber septum and the solution stirred under nitrogen for several minutes. In a separate vial, iBocCAF was added (130 mg, 2.7×10^{-4} mol), followed by dry DCM (1.3 mL). The solution was stirred under a gentle stream of nitrogen and cooled in an ice bath. The solution of **[$PEG_{\sim 64}$-NH_2]$_8$** was then taken up into a syringe and slowly added dropwise to the solution of iBocCAF. After addition was complete, the solution was left to react (stirring) over ice and nitrogen for 1 h and then left to equilibrate to ambient temperature for 15 min. The solution was then concentrated to half its original volume and then added dropwise to excess cold diethyl ether to precipitate the product, **[$PEG_{\sim 64}$-iBoc-caffeamide]$_8$**, **[$PEG_{\sim 64}$-iBoc-CAF]$_8$**, as a sticky residue. Precipitation was carried out twice to remove excess iBocCAF (mass yield: ~90%, % purity of end groups = 93%). Refer to Figure S25 for peak assignments. The ratio of integral values for peaks **h** and **j** was used to determine the purity of the end groups. Peak **j** are assigned to 2× isobutyl CH_3 protons of **[iBoc-PEG$_{\sim 64}$-carbamate]** arms which form as a by-product as a result of the reaction of the NH_2 groups of PEG with carbonate groups (Scheme S2).

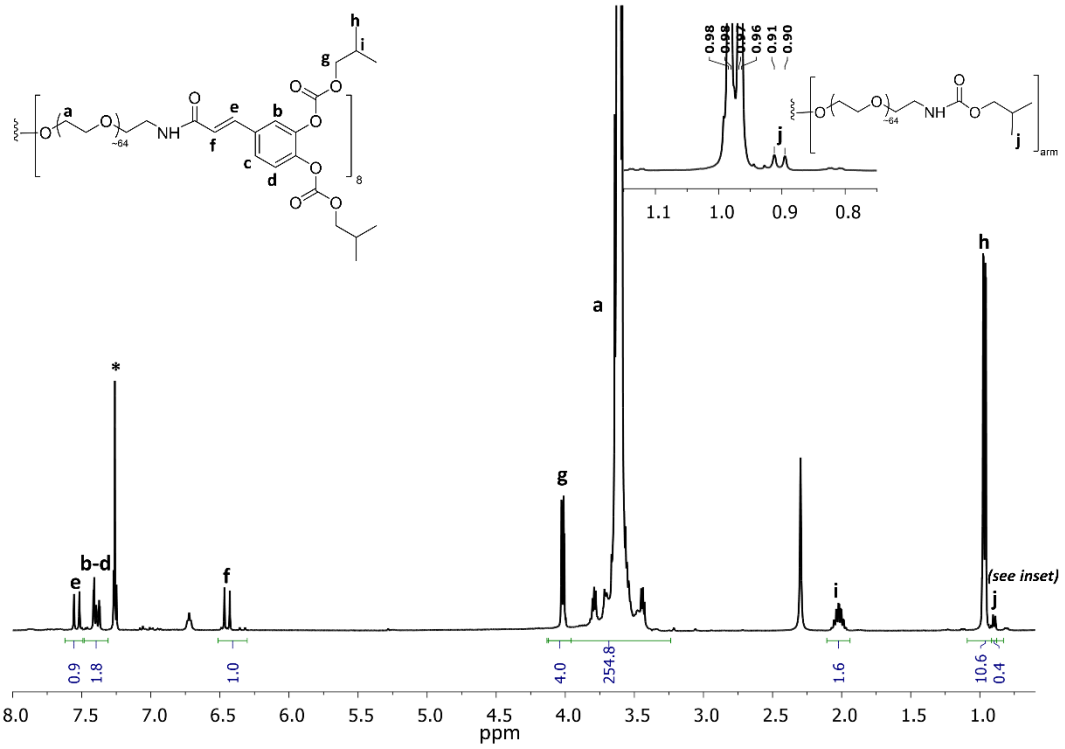


Figure S25. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 64}$-iBoc-caffeamide]$_8$, 8-arm PEG-iBocCAF prepared from [PEG$_{\sim 64}$-NH$_2$]$_8$ (~22 kDa). Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms which are a by-product of the reaction. *Residual NMR solvent peak.

[PEG$_{\sim 64}$-iBoc-caffeamide]$_8$ described above (440 mg, 2.7×10^{-5} mol) was dissolved in dry DCM (1.0 mL) in a vial. In a separate vial, a 32% v/v solution of isopropyl amine was prepared in dry DCM (360 µL of isopropylamine in 0.77 mL DCM). The PEG-iBocCAF solution was then added dropwise and slowly to the stirring isopropylamine/DCM solution. The final concentration of isopropylamine in DCM was ~17% v/v or 2.1 M, which is approximately 20 equivalents of isopropylamine per isobutyl carbonate group. The solution was stirred for 16 h at ambient temperature under N_2. The darkened solution was then concentrated to half its original volume and added dropwise to excess cold diethyl ether to precipitate the product, **[PEG$_{\sim 64}$-caffeamide]$_8$**, as a sticky tan residue. The solid was then dissolved in 0.1 M HCl buffer and dialyzed against acidic water for 3 days. The product was then freeze dried to isolate the product as a powder (cream) (yield: ~80%). Refer to Figure S26 for peak assignments. The 8-arm PEG-CAF was used for capsule fabrication and further referred to as **20 kDa 8-arm PEG-CAF** in capsule analysis.

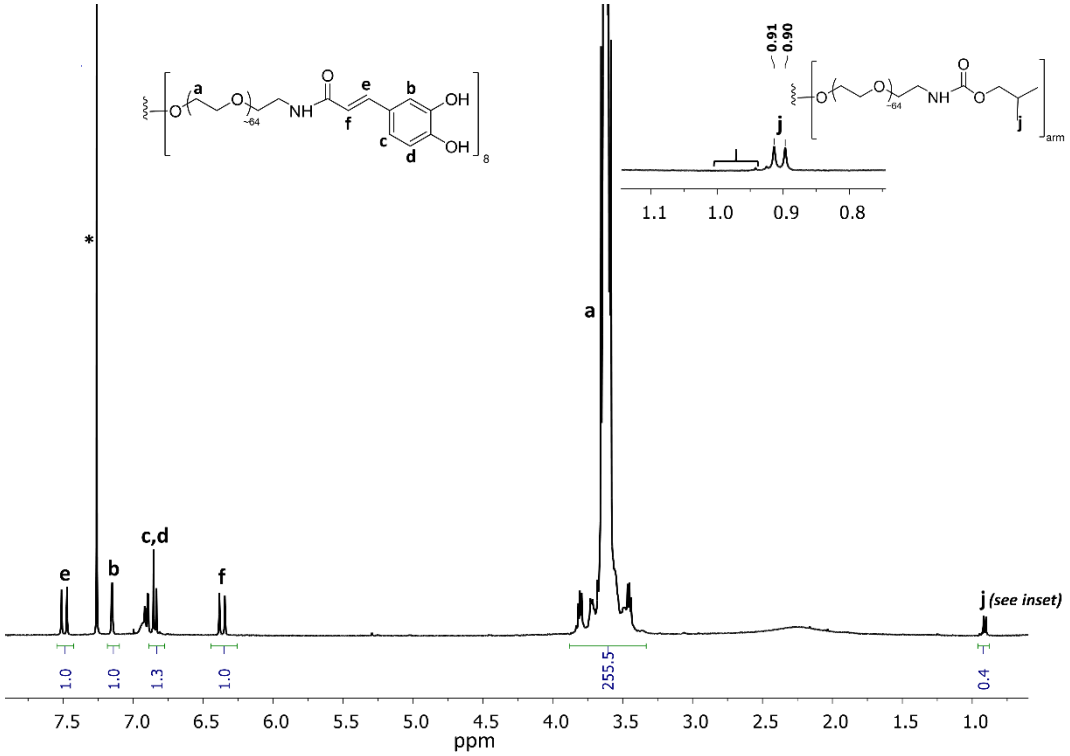


Figure S26. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of [PEG$_{\sim 64}$-caffeamide]$_8$, 8-arm PEG-CAF prepared from [PEG$_{\sim 64}$-iBoc-caffeamide]$_8$. Inset shows the absence of isobutoxy carbonyl (4× –CH_3) proton signals originally from the protected end groups. Peaks **j** in the inset are assigned to 2× isobutyl CH_3 protons of [iBoc-PEG carbamate] arms (by-product) which remain after purification. *Residual NMR solvent peak.

6. Synthesis of $CaCO_3$ Particles

A modified precipitation method was used to synthesize $CaCO_3$ templates (3.19 ± 0.34 μm) in aqueous solution in the presence of PSS (70 kDa) at room temperature according to a previously reported procedure.[1] Briefly, 1 M $CaCl_2$ (5 mL) and PSS (20 mL, 10 mg mL^{-1}) were added in water (175 mL), and the mixture was thoroughly stirred. A solution consisting of 1 M Na_2CO_3 (1 mL), PSS (0.5 mL, 10 mg mL^{-1}), and water (3.5 mL) was well mixed before addition to the above mixture. When the particle size reached the desired range, the PSS-stabilized $CaCO_3$ particles were separated through centrifugation (1000*g*, 1 min) and then washed with water. The particles were then dried in an oven at 80 °C for 24 h and then calcined in a furnace at 500 °C for 2 h to obtain the highly porous $CaCO_3$ particles.

7. Synthesis of PEG-Caffeamide–Fe^{III} MPN Capsules from $CaCO_3$ Particles

MPN capsules using $CaCO_3$ particles as sacrificial templates were prepared using a slightly different procedure from that used to fabricated MPN capsules using PS-COOH particle templates. A dispersion of $CaCO_3$ particles (3.19 ± 0.34 μm, 10 mg mL^{-1}) was sonicated for 15 min to disperse the particles in solution. A PEG-caffeamide stock solution and an $FeCl_3 \cdot 6H_2O$ solution were then added to obtain final concentrations of 0.5 mM and 2–8 mM (i.e., 2 mM for 2-arm PEG, 4 mM for 4-arm PEG, and 8 mM for 8-arm PEG-CAF), respectively, and then mixed by vortexing for 2 min. The final concentration of the particles in the assembly step was 2 mg mL^{-1}. Tris-HCl buffer (50 mM, pH 8.5, 0.7 mL) was then added to raise the pH to above 7 to form bis- and tris-coordination complexes between the PEG-caffeamide and Fe^{III}. Excess and unreacted materials were then removed by pelleting the particles (2000*g*, 2 min) and removing the supernatant. The MPN-coated particles were washed three times with water (500 μL) followed by repeated centrifugation (2000*g*, 2 min). The particles were resuspended in water (100 μL), after which EDTA solution (100 μL, 100 mM, pH 8.0) was added to remove the $CaCO_3$ templates. The MPN capsules were pelleted through centrifugation (3000*g*, 2 min) and washed with 500 mL water twice, followed by centrifugation (2000*g*, 2 min). The resulting PEG-caffeamide–Fe^{III} MPN capsules were dispersed in water (300 μL).

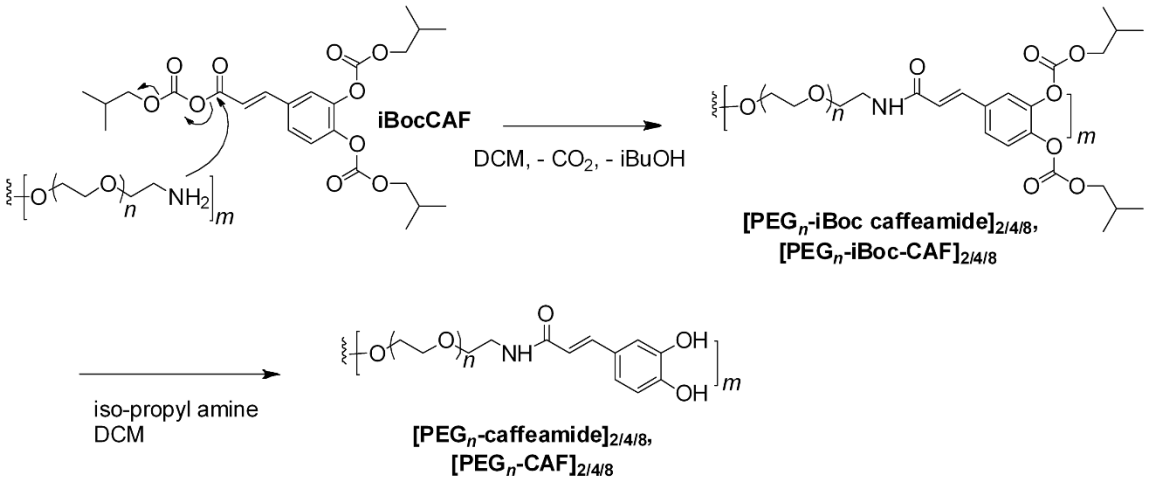
8. Thickness Comparison of MPN Capsules

The film thickness of the MPN capsules was analyzed by AFM using JPK SPM data processing software (v.5.0.13) and the average of three measurements was used to determine the mean shell thickness of each MPN capsule.

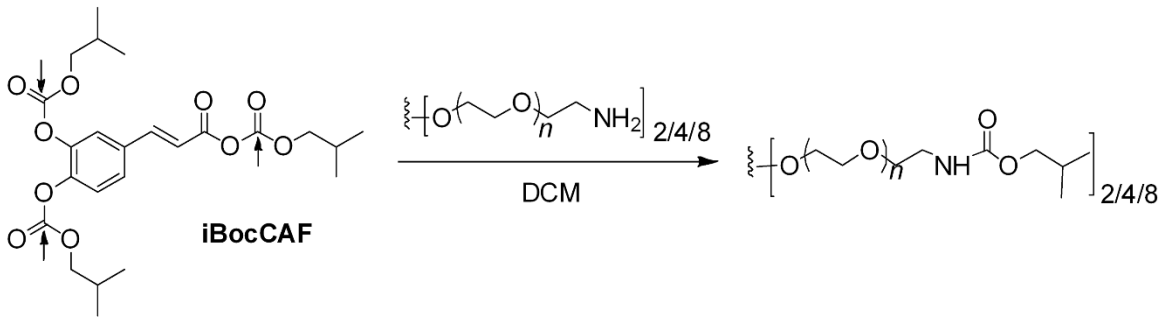
9. Synthesis of FITC-Dextran-Labeled $CaCO_3$ Particles and Fluorescent MPN Capsules

To prepare FITC-dextran-labeled $CaCO_3$ particles, FITC-$dextran_{2000\ kDa}$ solution (150 μL, 10 mg mL^{-1}) and $CaCO_3$ particles solution (150 μL, 30 mg mL^{-1}) were mixed and kept on a rotator at room

temperature for 16 h. The particles were then washed thrice with water by repeated centrifugation (2000*g*, 2 min) and finally dispersed in water (450 μL) to obtain 10 mg mL^{-1} FITC-dextran-labeled $CaCO_3$ particles. The fluorescent MPN capsules were prepared using the same procedure as that used for the fabrication of PEG-caffeamide–Fe^{III} MPN capsules using $CaCO_3$ particle templates.



Scheme S1. Synthesis of multi-arm PEG-caffeamide building blocks ($[PEG_n\text{-caffeamide}]_{2/4/8}$) from $[PEG_n\text{-}NH_2]_{2/4/8}$ and iBocCAF. Steps ii and iii from Scheme 1 (main manuscript) are shown here.



Scheme S2. Potential side reactions during the conjugation step (Step i in Scheme 1) resulting in the formation of $[\text{iBoc-PEG}_n\text{ carbamate}]_n$ arms as a result of the reaction of the amine groups of PEG with carbonate groups. Arrows indicate potential sites of amine attack, which results in the formation of a carbamate side product.

Table S1. Characteristics of synthesized PEG-caffeamide building blocks

Product[a]	M_n (g mol^{-1})[b]	Starting building block	Ratio[c]	Terminology[d]
[PEG$_{36}$-caffeamide]$_2$	2500	[PEG$_{30}$-NH$_2$]$_2$	1250:1	2.5 kDa 2-arm PEG-CAF
[PEG$_{90}$-caffeamide]$_2$	5000	[PEG$_{69}$-NH$_2$]$_2$	2500:1	5 kDa 2-arm PEG-CAF
[PEG$_{38}$-caffeamide]$_4$	5000	[PEG$_{32}$-NH$_2$]$_4$	1250:1	5 kDa 4-arm PEG-CAF
[PEG$_{64}$-caffeamide]$_4$	10000	[PEG$_{55}$-NH$_2$]$_4$	2500:1	10 kDa 4-arm PEG-CAF
[PEG$_{35}$-caffeamide]$_8$	10000	[PEG$_{32}$-NH$_2$]$_8$	1250:1	10 kDa 8-arm PEG-CAF
[PEG$_{64}$-caffeamide]$_8$	20000	[PEG$_{64}$-NH$_2$]$_8$	2500:1	20 kDa 8-arm PEG-CAF

[a]Refers to the final product composition based on ^{1}H NMR analysis. [b]Approximate molecular weight of PEG only in the PEG-caffeamide building block (approx. = M_n of PEG-NH$_2$ starting material). [c]Ratio of the total molecular weight of the PEG arms to the number of catechol groups (at the end of the PEG arms), i.e., PEG M_n: catechol ratio. [d]Terminology used in the main manuscript figures.

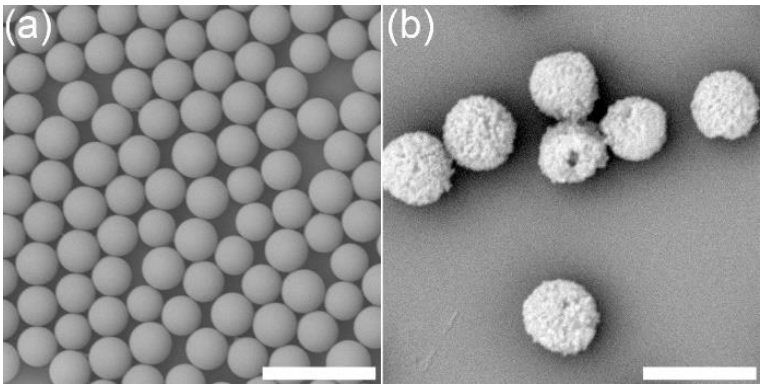


Figure S27. SEM images of sacrificial templates: (a) PS-COOH particles (1.86 ± 0.03 μm) and (b) $CaCO_3$ particles (3.19 ± 0.34 μm). Scale bars are 5 μm.

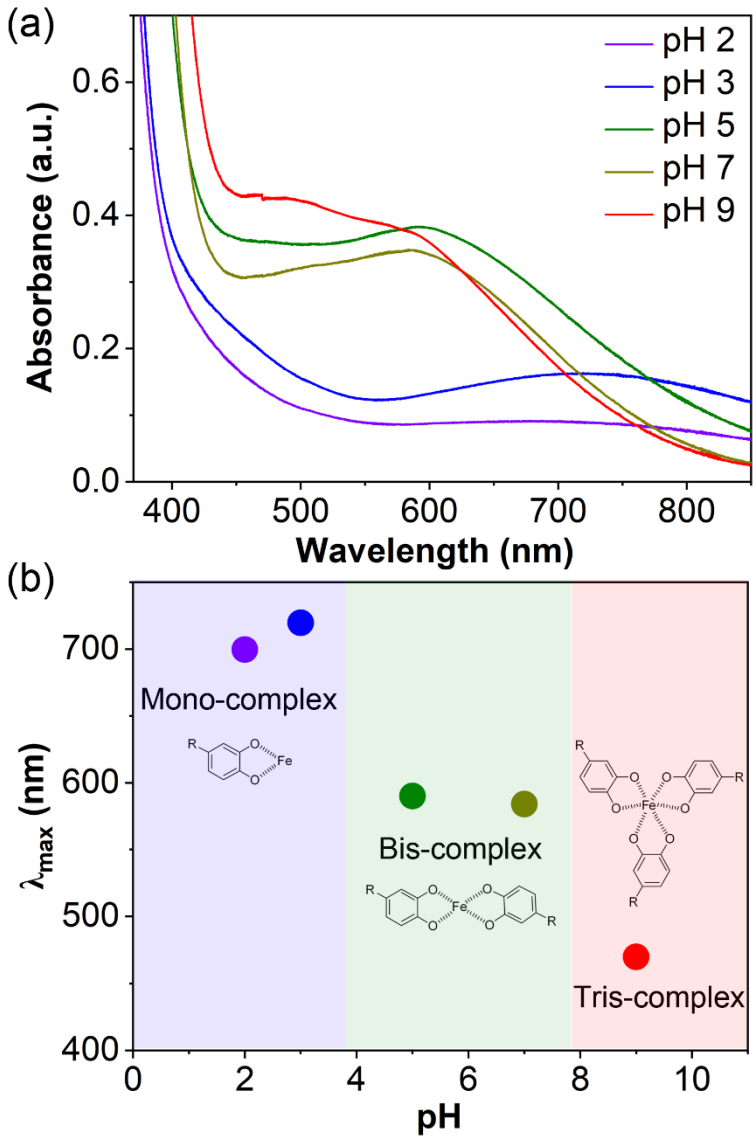


Figure S28. (a) UV–vis spectra and (b) absorbance maxima of the 10 kDa 4-arm PEG-caffeamide/Fe^{III} solutions at different pH (i.e., from pH 2 to 9). The three identified regions in (b) indicate the dominant mono-, bis-, and tris-complexes between PEG-caffeamides and Fe^{III} ions.

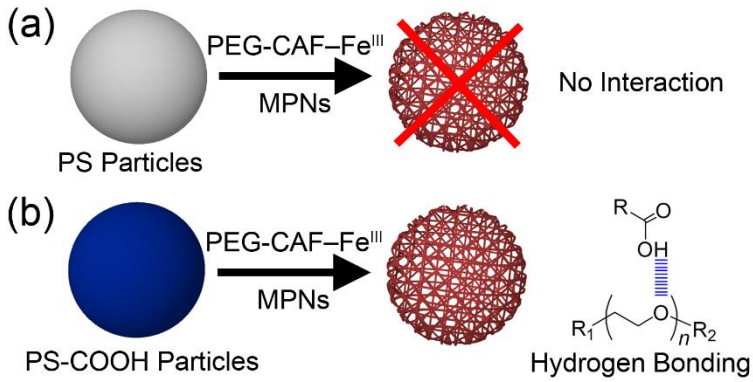


Figure S29. Preparation of PEG-caffeamide–Fe^{III} MPN capsules on (a) PS and (b) PS-COOH particles. MPN films only deposit onto the surface of PS-COOH particles as a result of hydrogen bonding interactions between the carboxylic acid groups of the particle surface and the backbone of PEG.

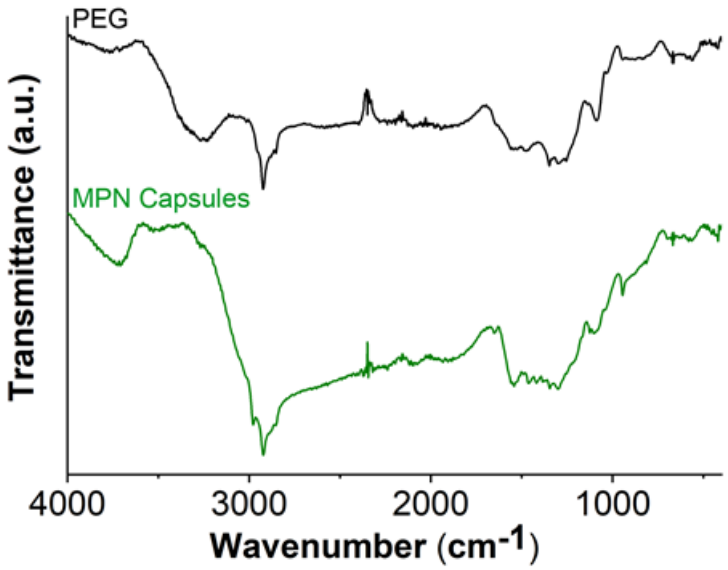


Figure S30. FTIR spectra of 10 kDa 4-arm PEG-caffeamide (black curve) and 10 kDa 4-arm PEG-caffeamide–Fe^{III} MPN capsules prepared using PS-COOH templates (green curve).

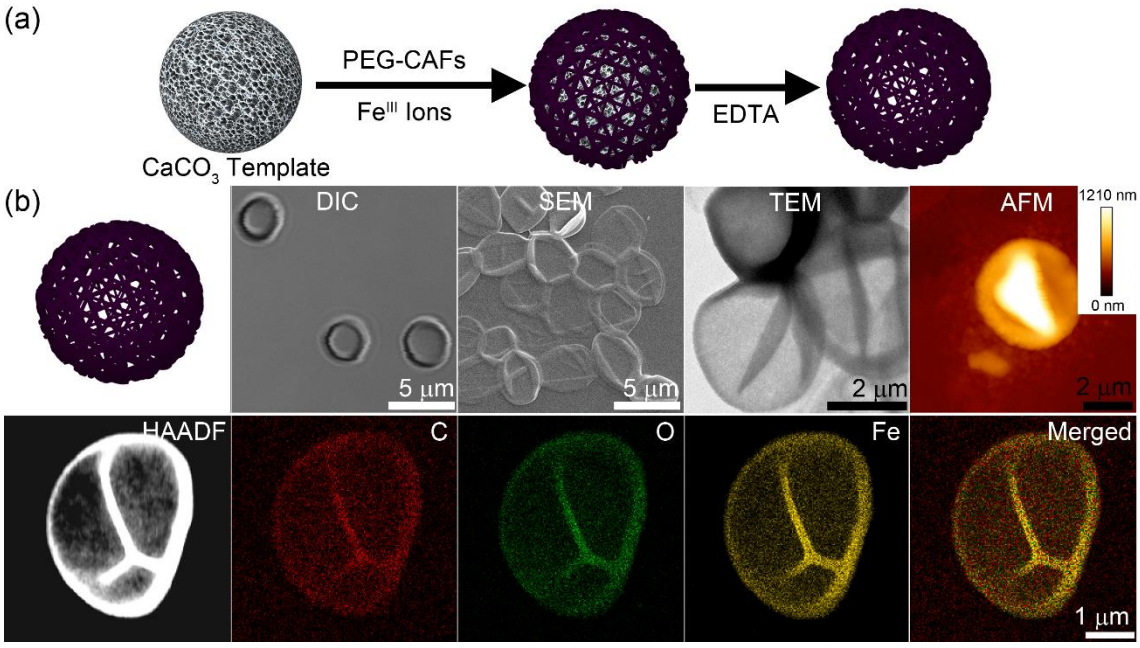


Figure S31. (a) Schematic of the preparation of PEG-caffeamide–Fe^{III} MPN capsules using $CaCO_3$ templates. (b) Characterization of 10 kDa 4-arm PEG-caffeamide–Fe^{III} MPN capsules derived from $CaCO_3$ sacrificial particles by DIC, SEM, TEM, AFM, HAADF, and EDX elemental mapping.

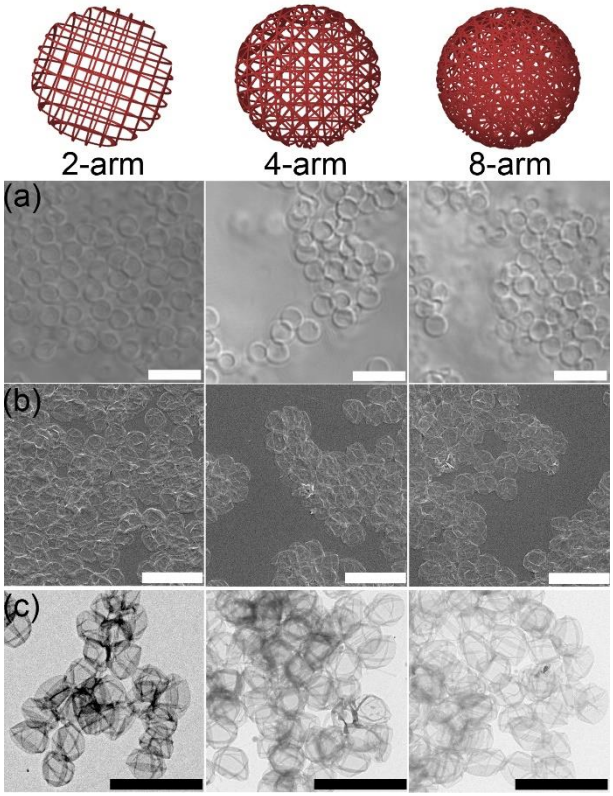
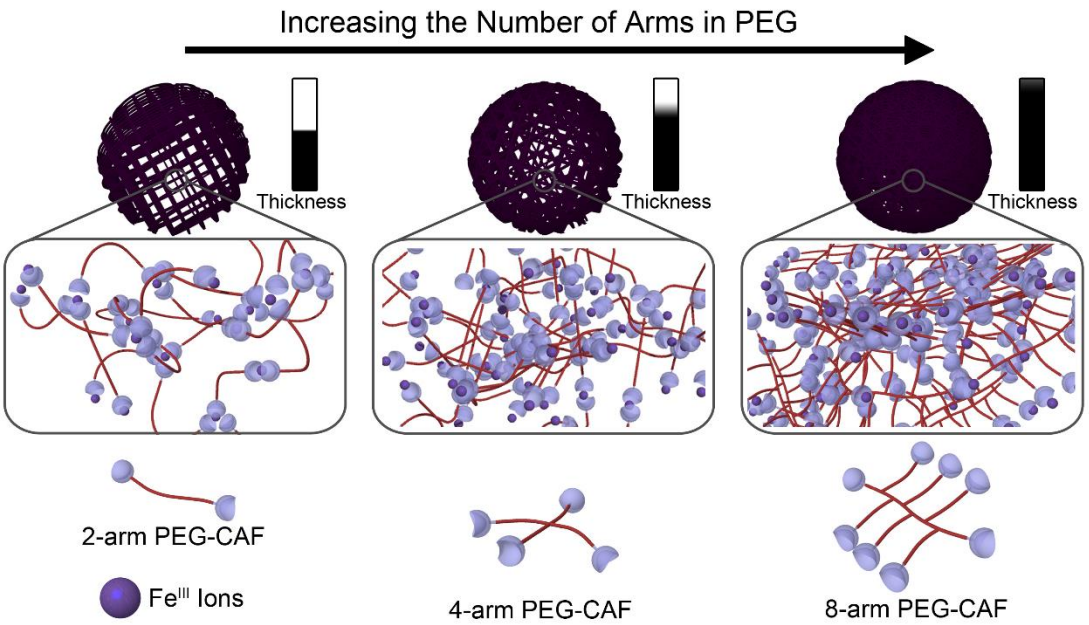


Figure S32. Microscopy images of PEG-caffeamide–Fe^{III} MPN capsules prepared using 5 kDa 2-arm, 10 kDa 4-arm, and 20 kDa 8-arm PEG-CAF building blocks and PS-COOH templates: (a) DIC, (b) SEM, and (c) TEM. All scale bars are 5 μm.



Scheme S3. PEG-caffeamide–Fe^{III} MPN capsules prepared from the coordination-driven cross-linking of Fe^{III} ions and different 2-, 4-, or 8-arm PEG-CAF building blocks on $CaCO_3$ templates.

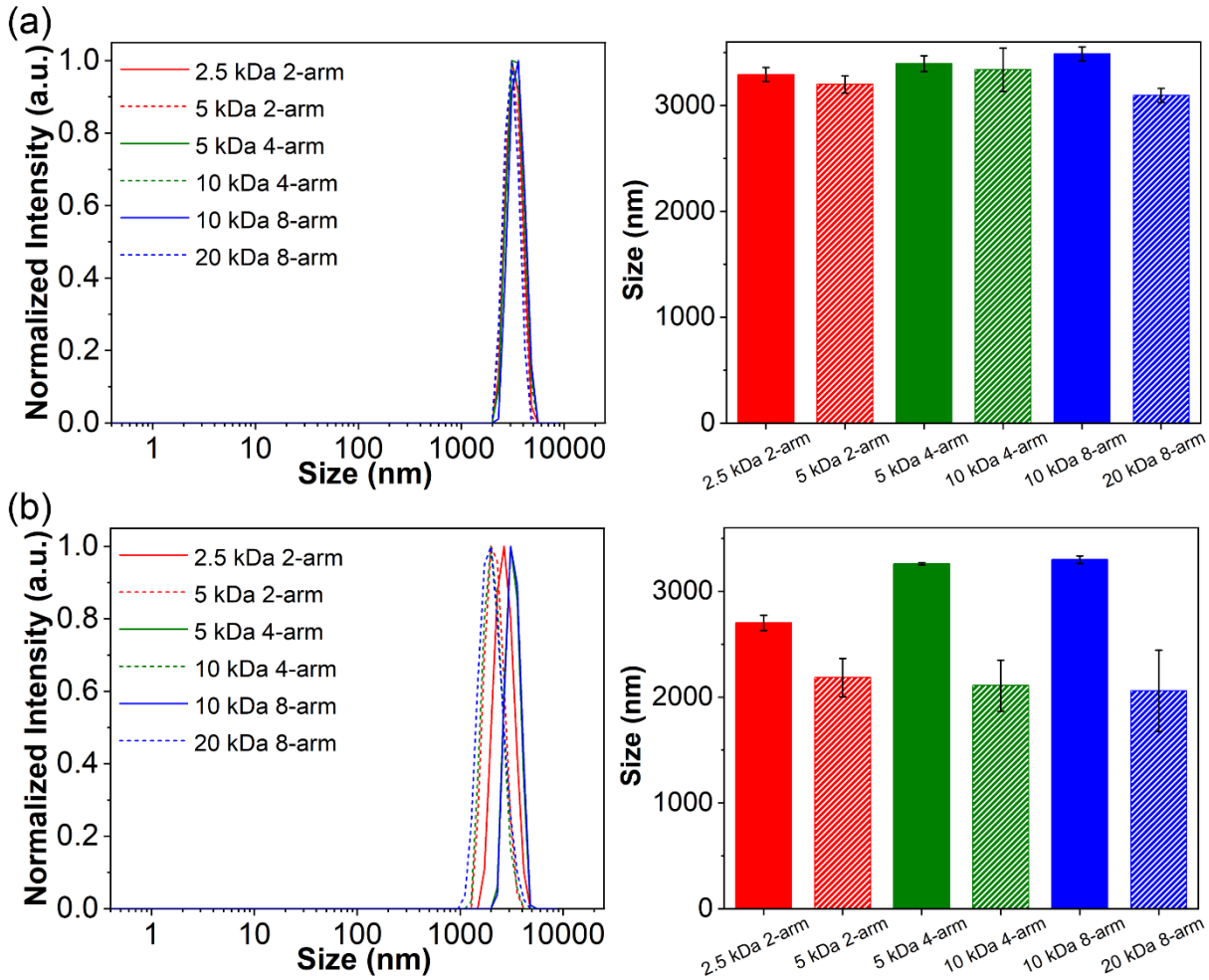


Figure S33. Dynamic light scattering (DLS) analysis of PEG-caffeamide–Fe^{III} MPN capsules prepared using (a) PS-COOH or (b) $CaCO_3$ templates. The hydrodynamic diameters, determined by DLS (intensity), are shown as the average ± standard deviation (SD) of three measurements.

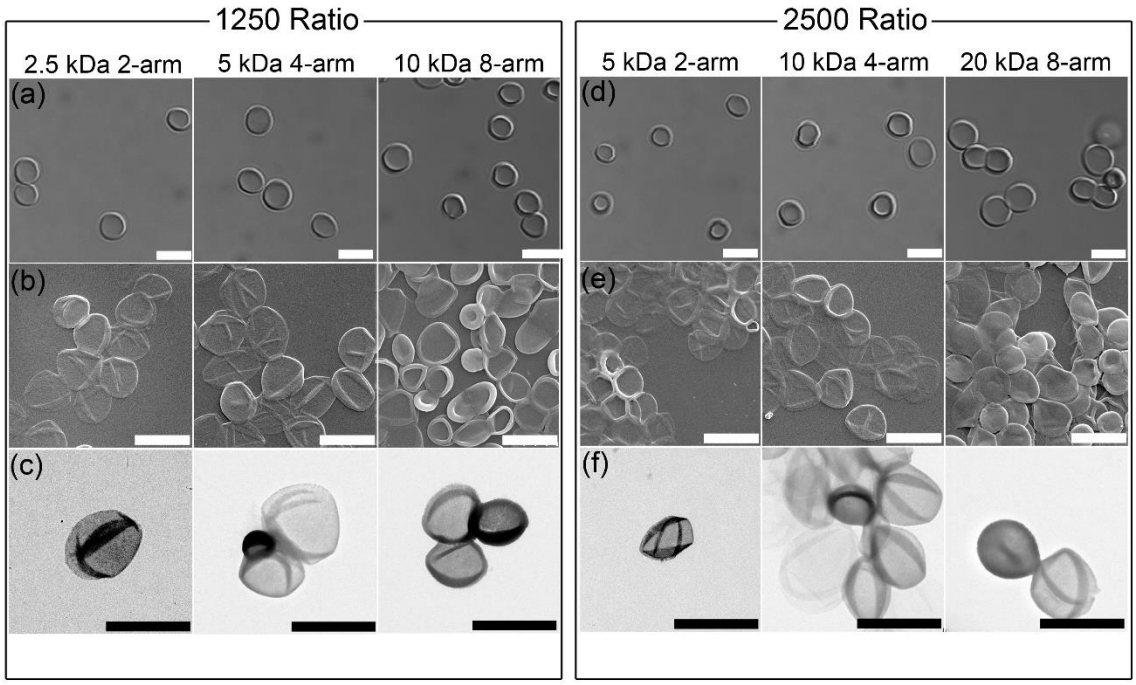


Figure S34. Microscopy images of PEG-caffeamide–Fe^{III} MPN capsules prepared using $CaCO_3$ templates and 2.5 kDa 2-arm, 5 kDa 4-arm, and 10 kDa 8-arm PEG-CAF building blocks (PEG M_n:catechol = 1250:1) and 5 kDa 2-arm, 10 kDa 4-arm, and 20 kDa 8-arm PEG-CAF building blocks (2500:1): (a,d) DIC, (b,e) SEM, and (c,f) TEM. Scale bars are 5 μm.

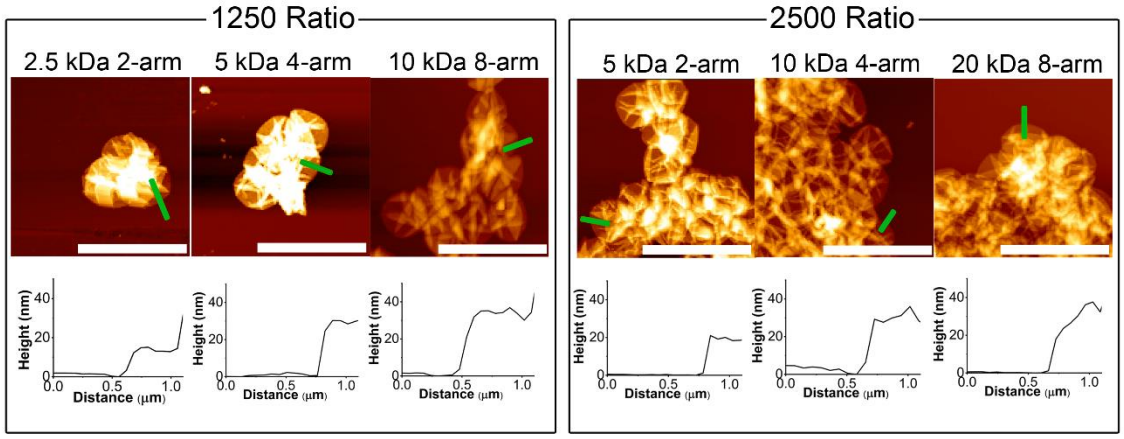


Figure S35. AFM images of PEG-caffeamide–Fe^{III} MPN capsules prepared using PS-COOH templates and 2-, 4-, and 8-arm PEG-CAF building blocks (PEG M_n:catechol = 1250:1 or 2500:1). Height–distance graphs corresponding to the green line in the AFM images are shown. Scale bars are 5 μm.

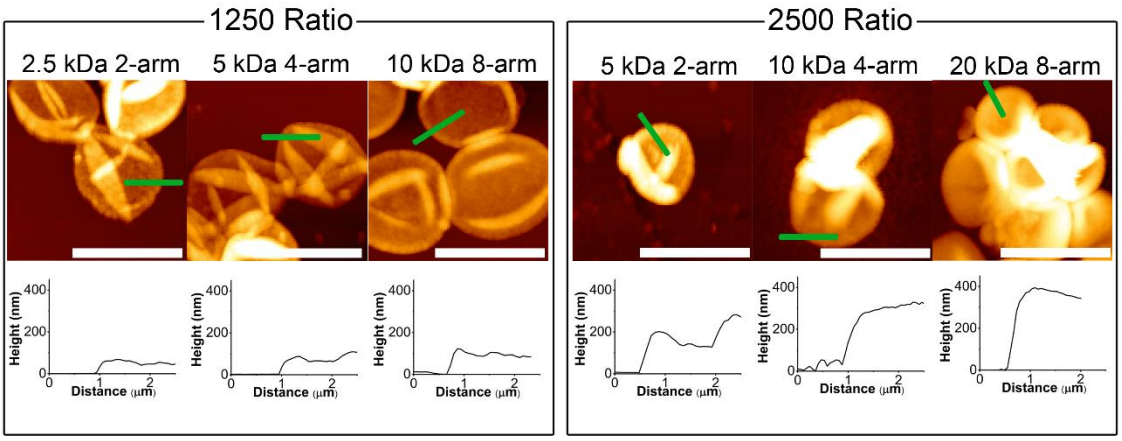


Figure S36. AFM images of PEG-caffeamide–Fe^{III} MPN capsules using $CaCO_3$ templates and 2-, 4-, and 8-arm PEG-CAF building blocks (PEG M_n:catechol = 1250:1 or 2500:1). Height–distance graphs corresponding to the green line in the AFM data are shown. Scale bars are 5 μm.

Table S2. Shell thickness of PEG-caffeamide–Fe^{III} MPN capsules prepared from PS-COOH and $CaCO_3$ templates

PEG-caffeamide building block	Shell thickness[a] (nm)	Shell thickness[b] (nm)
2.5 kDa 2-arm PEG-CAF	8.7 ± 2.0	27.6 ± 2.7
5 kDa 2-arm PEG-CAF	10.8 ± 1.1	73.4 ± 10.1
5 kDa 4-arm PEG-CAF	14.3 ± 0.1	39.4 ± 6.1
10 kDa 4-arm PEG-CAF	15.6 ± 1.5	162.9 ± 11.2
10 kDa 8-arm PEG-CAF	16.3 ± 1.1	59.0 ± 6.1
20 kDa 8-arm PEG-CAF	16.6 ± 1.6	169.3 ± 18.3

[a]Determined for capsules prepared from PS-COOH particle templates. [b]Determined for capsules prepared from $CaCO_3$ particle templates.

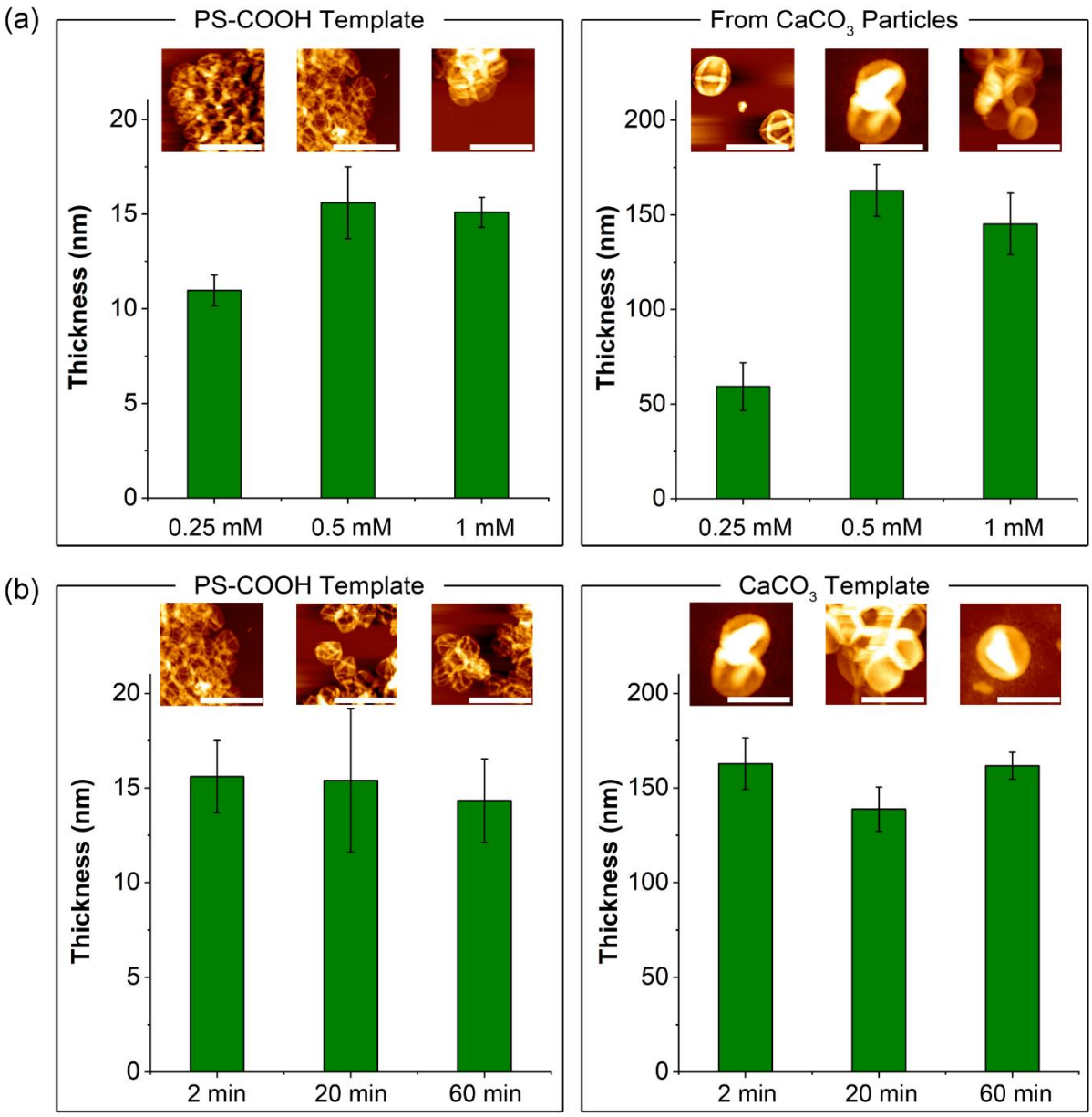


Figure S37. Effect of (a) PEG-caffeamide concentration in the assembly solution and (b) incubation time on the thickness of PEG-caffeamide–Fe^{III} MPN capsules. The effect of the concentration of PEG-caffeamide (10 kDa 4-arm PEG-caffeamide) was examined by keeping the catechol/Fe^{III} ion ratio as 1:1 when PS-COOH templates were used and 1:2 when $CaCO_3$ templates were used. To examine the effect of incubation time, the template particles were incubated in 0.5 mM 4-arm PEG-CAF and 2 mM $FeCl_3 \cdot 6H_2O$ solution for the PS-COOH template system and in 0.5 mM 4-arm PEG-CAF and 4 mM $FeCl_3 \cdot 6H_2O$ solution for the $CaCO_3$ template system. The thickness was determined from height–distance AFM graphs and the data are shown as the mean ± SD of three independent AFM measurements. Scale bars are 5 µm.

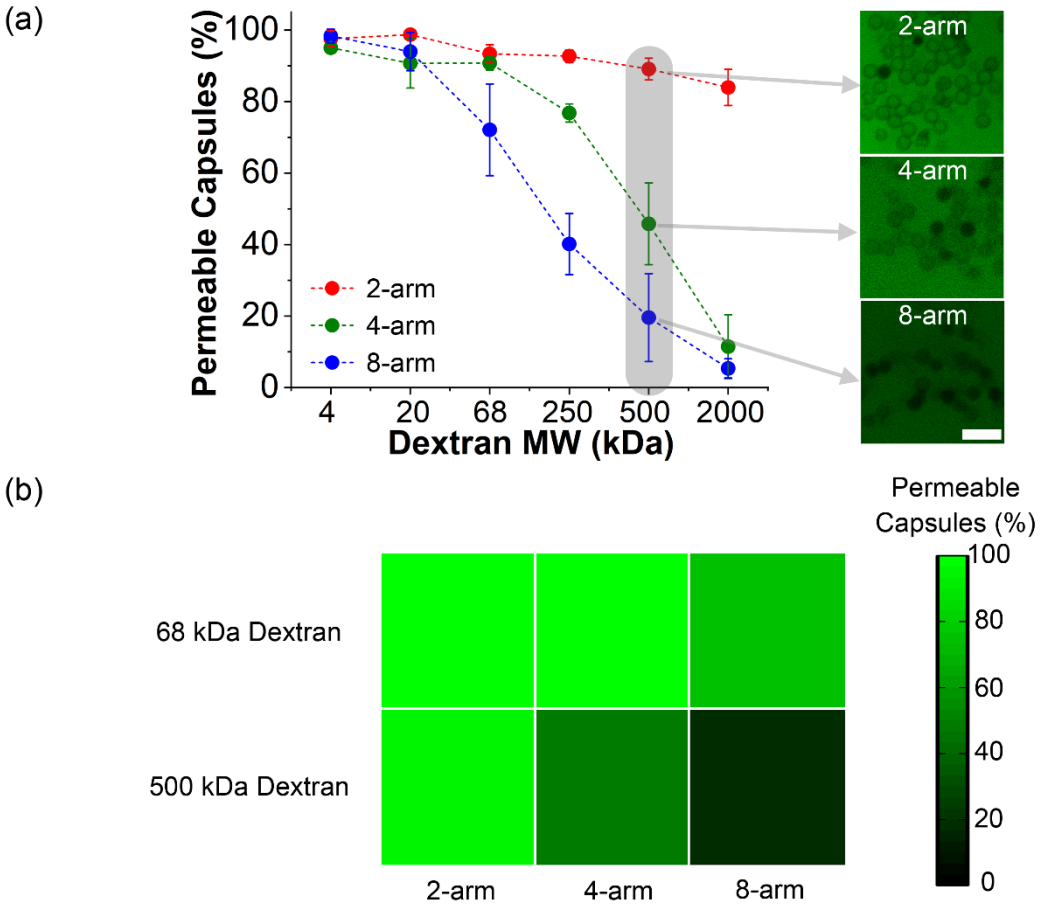


Figure S38. (a) Comparison of the permeability of PEG-caffeamide–Fe^{III} MPN capsules prepared using 5 kDa 2-arm, 10 kDa 4-arm, and 20 kDa 8-arm PEG-CAF building blocks and PS-COOH templates against FITC-dextran with MW ranging from 4 to 2000 kDa. At a given PEG M_n-to-catechol ratio (2500:1), varying the number of PEG arms (2-, 4-, and 8-arm) influences the permeability of the capsules. Scale bars are 5 μm. Error bars represent the SD of three independent experiments. (b) Heat map showing the percentage of capsules permeable to 68 kDa and 500 kDa FITC-dextran.

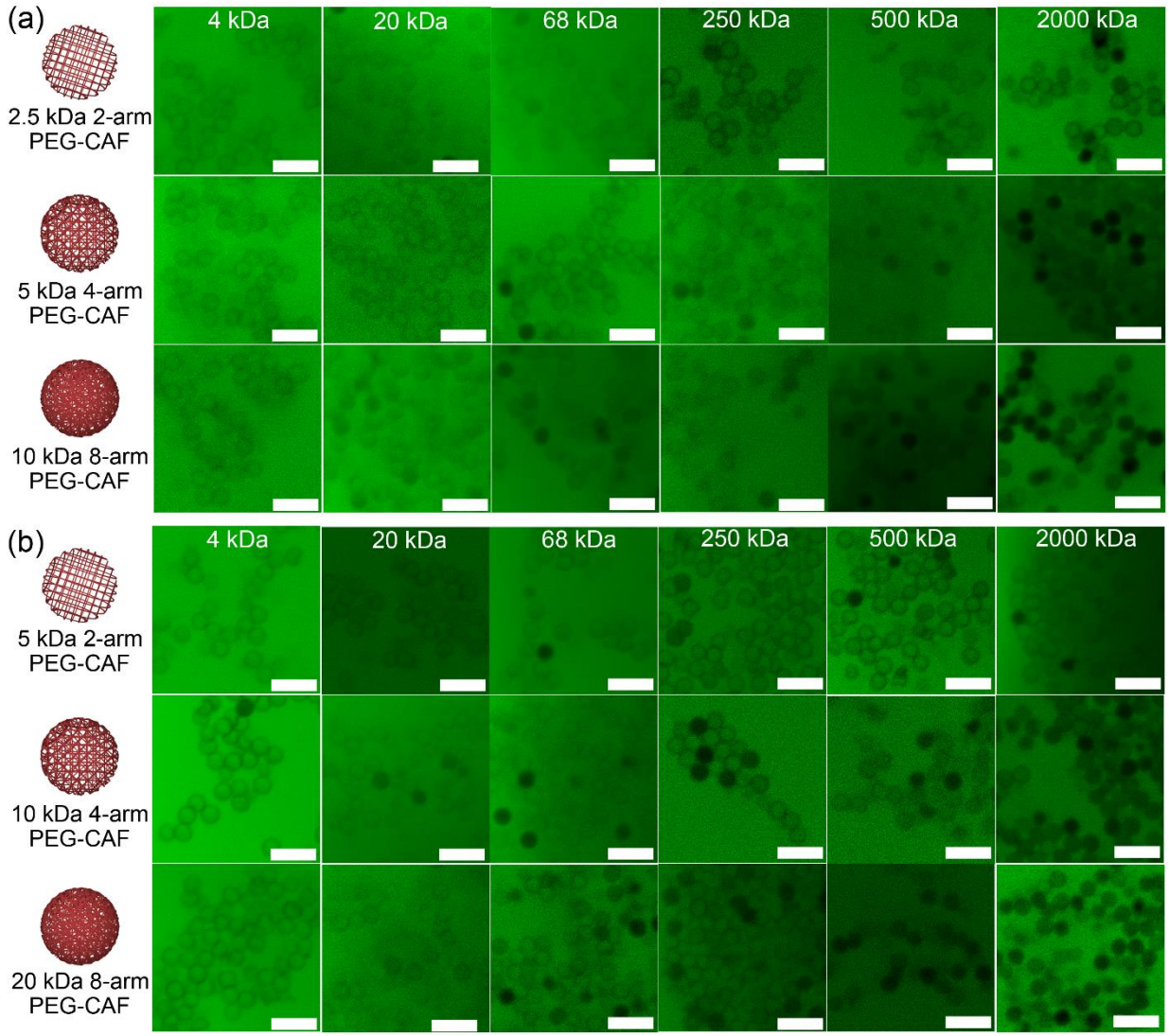


Figure S39. Comparison of the permeability of 2-, 4-, and 8-arm PEG-caffeamide–Fe^{III} MPN capsules prepared from PS-COOH templates against FITC-dextran with MW ranging from 4 to 2000 kDa. In (a) and (b), the PEG M_n-to-catechol ratios were 1250:1 and 2500:1, respectively. Scale bars are 5 μm.

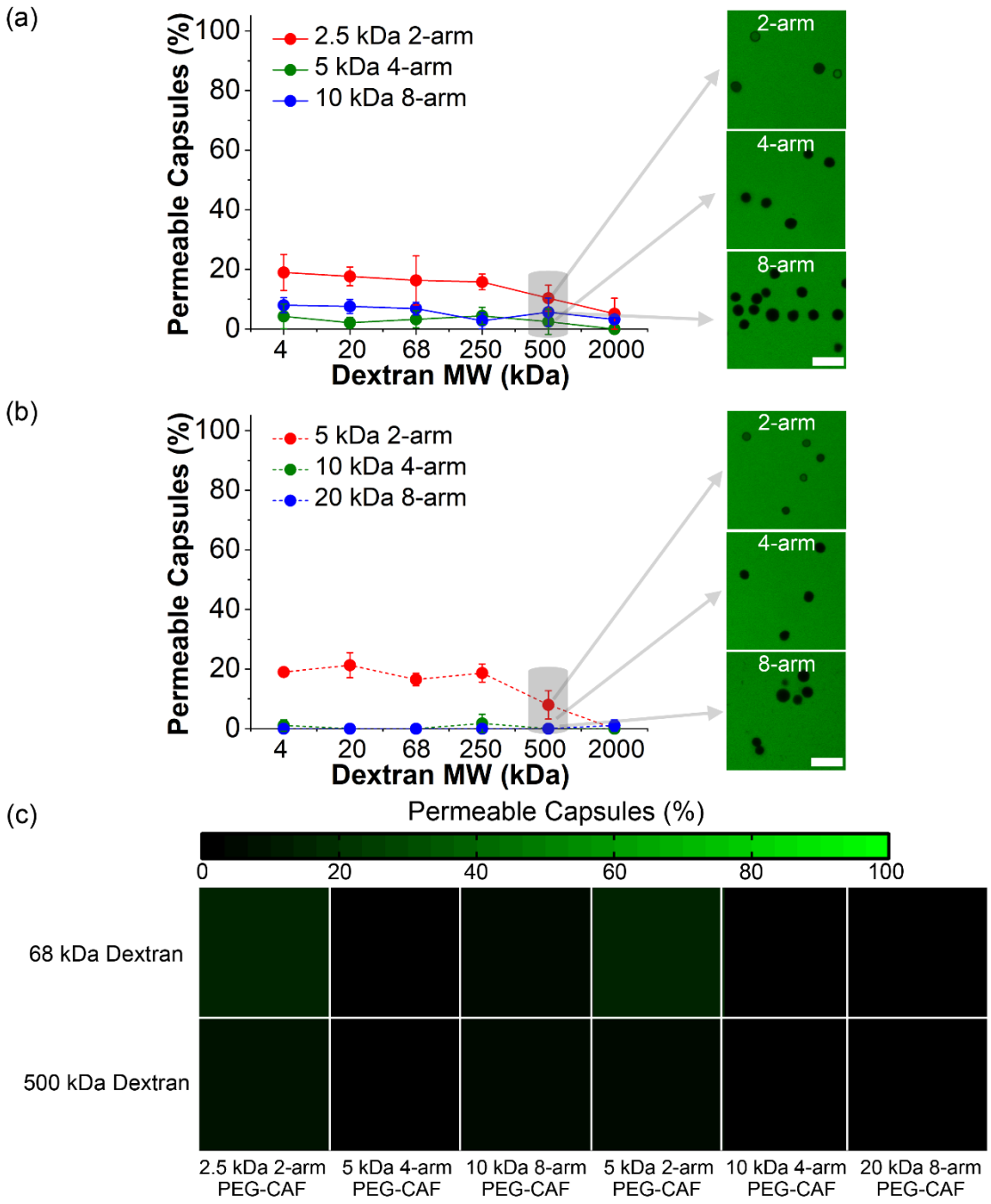


Figure S40. Comparison of the permeability of PEG-caffeamide–Fe^{III} MPN capsules using $CaCO_3$ templates and (a) 2.5 kDa 2-arm, 5 kDa 4-arm, and 10 kDa 8-arm PEG-CAF building blocks at a PEG M_n-to-catechol ratio of 1250:1 or (b) 5 kDa 2-arm, 10 kDa 4-arm, and 20 kDa 8-arm PEG-CAF building blocks at a PEG M_n-to-catechol ratio of 2500:1 against FITC-dextran with MW ranging from 4 to 2000 kDa. A significant reduction in permeability (<25%) is apparent throughout the series examined. Scale bars are 5 μm. Error bars represent the SD of three independent experiments. (b) Heat map showing the percentage of capsules permeable to 68 kDa and 500 kDa FITC-dextran.

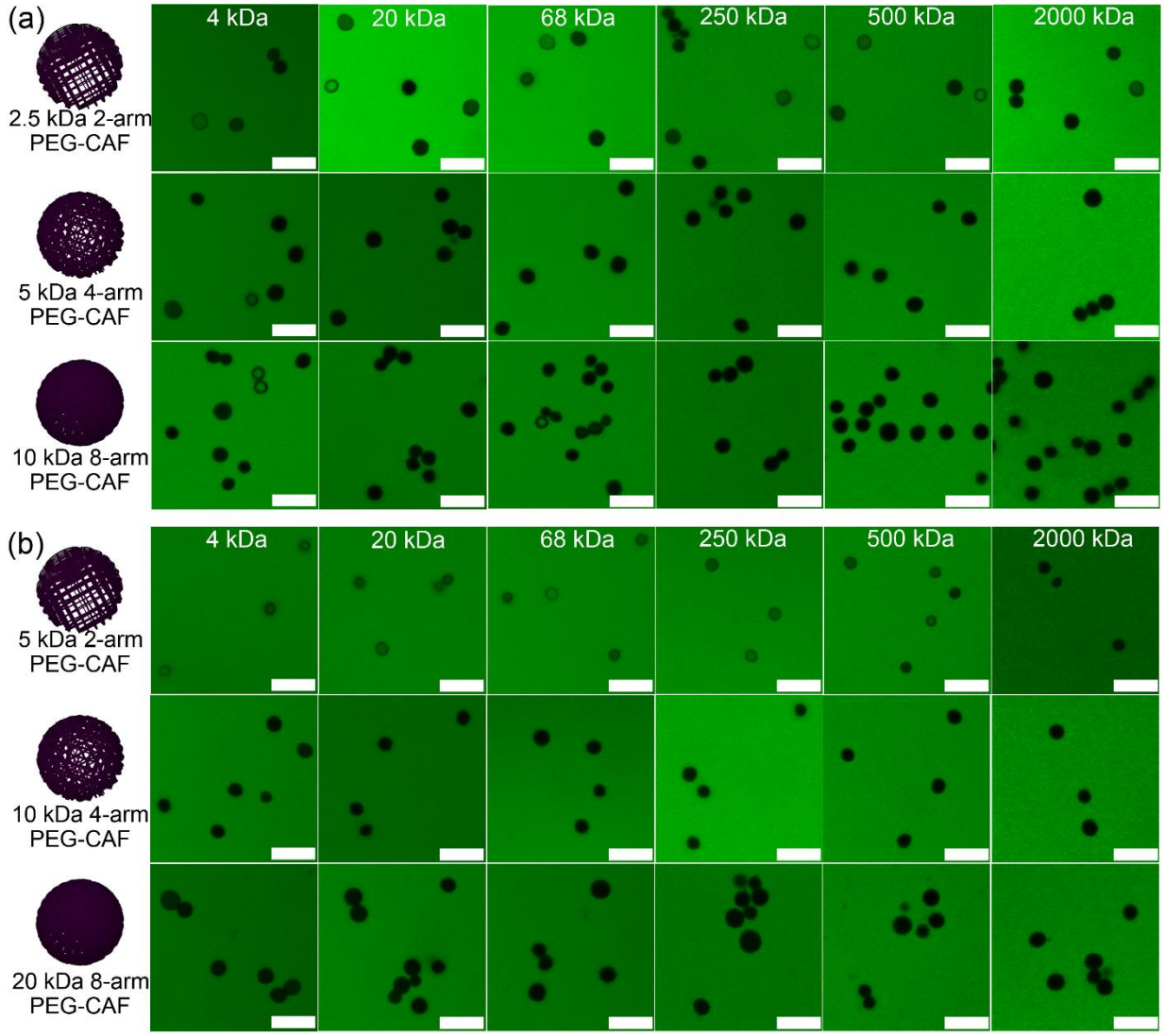


Figure S41. Comparison of permeability of 2-, 4-, and 8-arm PEG-caffeamide–Fe^{III} MPN capsules using $CaCO_3$ templates and (a) 2.5 kDa 2-arm, 5 kDa 4-arm, and 10 kDa 8-arm PEG-CAF building blocks at a PEG M_n-to-catechol ratio of 1250:1 or (b) 5 kDa 2-arm, 10 kDa 4-arm, and 20 kDa 8-arm PEG-CAF building blocks at a PEG M_n-to-catechol ratio of 2500:1 against FITC-dextran with MW ranging from 4 to 2000 kDa. Scale bars are 10 μm.

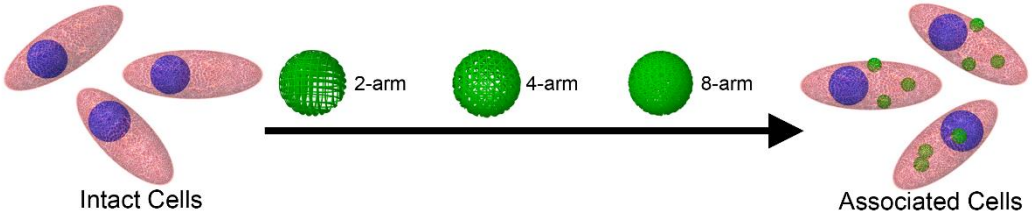


Figure S42. Schematic illustration of the cell association experiments conducted with different PEG-caffeamide–Fe^{III} MPN capsules having green fluorescence. Cell membranes and cell nuclei were stained with WGA594 (red) and Hoechst 33342 (blue), respectively. The capsules were fluorescently labeled (green) for visualization.

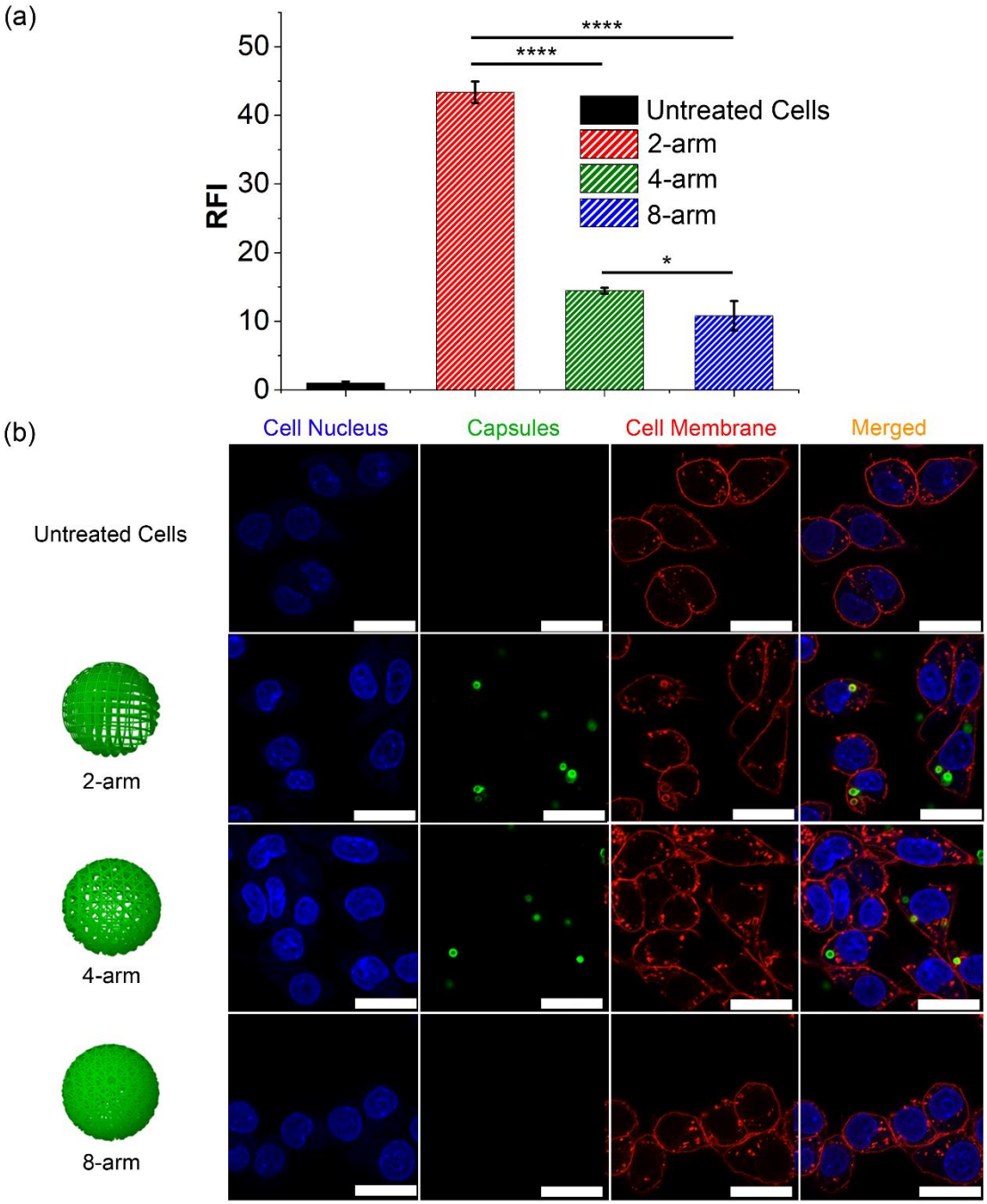


Figure S43. (a) Flow cytometry analysis of RAW 264.7 cells associated with fluorescent PEG-caffeamide–Fe^{III} MPN capsules prepared from 5 kDa 2-arm, 10 kDa 4-arm, or 20 kDa 8-arm PEG-CAF building blocks; incubation was carried out for 4 h at 37 °C. Bars with stripes correspond to capsules prepared from PEG-caffeamide building blocks with a PEG M_n-to-catechol ratio of 2500:1. Relative fluorescence intensity (RFI) to untreated cells is shown. Error bars represent the SD of three independent experiments. Statistical significance was determined by one-way ANOVA analysis: **** $p < 0.0001$ and * $p < 0.05$. (b) CLSM images of RAW 264.7 cells incubated with fluorescent PEG-caffeamide–Fe^{III} MPN capsules prepared from 5 kDa 2-arm, 10 kDa 4-arm, or 20 kDa 8-arm PEG-CAF building blocks (incubation time of 4 h at 37 °C). Cell membranes and nuclei were stained with WGA594 (red) and Hoechst 33342 (blue), respectively. Green fluorescence represents fluorescent MPN capsules. Scale bars are 20 µm.

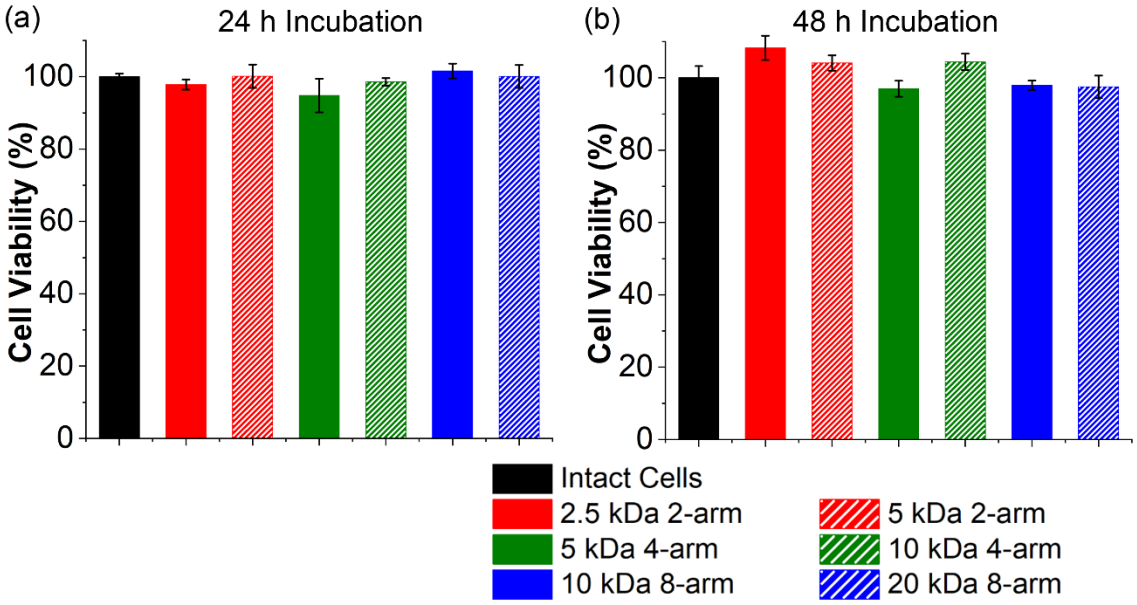


Figure S44. Cytotoxicity of PEG-caffeamide–Fe^{III} MPN capsules toward RAW 264.7 cells following incubation for (a) 24 h and (b) 48 h in DMEM with 10% FBS at 37 °C. Cell viability was evaluated by XTT assay (mean ± SD, n = 3). The cell viability of untreated cells (black) was normalized to 100%. Bars with solid colors correspond to capsules prepared from PEG-caffeamide building blocks with a PEG M_n-to-catechol ratio of 1250:1 and bars with diagonal stripes correspond to capsules prepared from PEG-caffeamide building blocks with a PEG M_n-to-catechol ratio of 2500:1. The number of PEG-caffeamide–Fe^{III} MPN capsules and cells were 8.0×10^5 and 8.0×10^3, respectively, corresponding to a capsule-to-cell ratio of 100:1.

REFERENCES

(1) Volodkin, D. V.; Larionova, N. I.; Sukhorukov, G. B. Protein Encapsulation Via Porous $CaCO_3$ Microparticles Templating. *Biomacromolecules* **2004**, *5*, 1962–1972.

Checklist

Minimum Information Reporting in Bio–Nano Experimental Literature

The MIRIBEL guidelines were introduced here: https://doi.org/10.1038/s41565-018-0246-4

The development of these guidelines was led by the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology: https://www.cbns.org.au/. Any updates or revisions to this document will be made available here: http://doi.org/10.17605/OSF.IO/SMVTF. This document is made available under a CC-BY 4.0 license: https://creativecommons.org/licenses/by/4.0/.

The MIRIBEL guidelines were developed to facilitate reporting and dissemination of research in bio–nano science. Their development was inspired by various similar efforts:

- MIAME (microarray experiments): *Nat. Genet.* **29** (2001), 365; http://doi.org/10.1038/ng1201-365
- MIRIAM (biochemical models): *Nat. Biotechnol.* **23** (2005) 1509; http://doi.org/10.1038/nbt1156
- MIBBI (biology/biomedicine): *Nat. Biotechnol.* **26** (2008) 889; http://doi.org/10.1038/nbt.1411
- MIGS (genome sequencing): *Nat. Biotechnol.* **26** (2008) 541; http://doi.org/10.1038/nbt1360
- MIQE (quantitative PCR): *Clin. Chem.* **55** (2009) 611; http://doi.org/10.1373/clinchem.2008.112797
- ARRIVE (animal research): *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412
- *Nature*'s reporting standards:
    - Life science: https://www.nature.com/authors/policies/reporting.pdf; e.g., *Nat. Nanotechnol.* **9** (2014) 949; http://doi.org/10.1038/nnano.2014.287
    - Solar cells: https://www.nature.com/authors/policies/solarchecklist.pdf; e.g., *Nat. Photonics* **9** (2015) 703; http://doi.org/10.1038/nphoton.2015.233
    - Lasers: https://www.nature.com/authors/policies/laserchecklist.pdf; e.g., *Nat. Photonics* **11** (2017) 139; http://doi.org/10.1038/nphoton.2017.28
- The "TOP guidelines": e.g., *Science* **352** (2016) 1147; http://doi.org/10.1126/science.aag2359

Similar to many of the efforts listed above, the parameters included in this checklist are **not** intended to be definitive requirements; instead they are intended as 'points to be considered', with authors themselves deciding which parameters are—and which are not—appropriate for their specific study.

This document is intended to be a living document, which we propose is revisited and amended annually by interested members of the community, who are encouraged to contact the authors of this document. Parts of this document were developed at the annual International Nanomedicine Conference in Sydney, Australia: http://www.oznanomed.org/, which will continue to act as a venue for their review and development, and interested members of the community are encouraged to attend.

After filling out the following pages, this checklist document can be attached as a "Supporting Information" document during submission of a manuscript to inform Editors and Reviewers (and eventually readers) that all points of MIRIBEL have been considered.

Supplementary Table 1. Material characterization*

Question	Yes	No
1.1 Are "**best reporting practices**" **available** for the nanomaterial used? For examples, see *Chem. Mater.* **28** (2016) 3535; http://doi.org/10.1021/acs.chemmater.6b01854 and *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235		√
1.2 If they are available, **are they used**? If not available, ignore this question and proceed to the next one.		
1.3 Are extensive and clear instructions reported detailing all steps of **synthesis** and the resulting **composition** of the nanomaterial? For examples, see *Chem. Mater.* **26** (2014) 1765; http://doi.org/10.1021/cm500632c, and *Chem. Mater.* **26** (2014) 2211; http://doi.org/10.1021/cm5010449. Extensive use of photos, images, and videos are strongly encouraged. For example, see *Chem. Mater.* **28** (2016) 8441; http://doi.org/10.1021/acs.chemmater.6b04639	√	
1.4 Is the **size** (or **dimensions**, if non-spherical) and **shape of** the nanomaterial reported?	√	
1.5 Is the **size dispersity** or **aggregation** of the nanomaterial reported?	√	
1.6 Is the **zeta potential** of the nanomaterial reported?		√
1.7 Is the **density (mass/volume)** of the nanomaterial reported?		√
1.8 Is the amount of any **drug loaded** reported? 'Drug' here broadly refers to functional cargos (e.g., proteins, small molecules, nucleic acids).	not applicable	
1.9 Is the **targeting performance** of the nanomaterial reported, including **amount** of ligand bound to the nanomaterial if the material has been functionalised through addition of targeting ligands?	not applicable	
1.10 Is the **label signal** per nanomaterial/particle reported? For example, fluorescence signal per particle for fluorescently labelled nanomaterials.		√
1.11 If a material property not listed here is varied, has it been **quantified**?		√
1.12 Were characterizations performed in a **fluid mimicking biological conditions**?		√
1.13 Are details of how these parameters were **measured/estimated** provided?	√	
Explanation for **No** (if needed): 1.12: Particles were characterized by DIC microscopy in aqueous solution.		

*****Ideally, material characterization should be performed in the same biological environment as that in which the study will be conducted. For example, for cell culture studies with nanoparticles, characterization steps would ideally be performed on nanoparticles dispersed in cell culture media. If this is not possible, then characteristics of the dispersant used (e.g., pH, ionic strength) should mimic as much as possible the biological environment being studied.

Supplementary Table 2. Biological characterization*

Question	Yes	No
2.1 Are **cell seeding details**, including **number of cells plated, confluency at start of experiment,** and **time between seeding and experiment** reported?	√	
2.2 If a standardised cell line is used, are the **designation and source** provided?	√	
2.3 Is the **passage number** (total number of times a cell culture has been subcultured) known and reported?	√	
2.4 Is the last instance of **verification of cell line** reported? If no verification has been performed, is the time passed and passage number since acquisition from trusted source (e.g., ATCC or ECACC) reported? For information, see *Science* **347** (2015) 938; http://doi.org/10.1126/science.347.6225.938		√
2.5 Are the results from **mycoplasma testing** of cell cultures reported?	√	
2.6 Is the **background signal of cells**/**tissue** reported? (E.g., the fluorescence signal of cells without particles in the case of a flow cytometry experiment.)	√	
2.7 Are **toxicity studies** provided to demonstrate that the material has the expected toxicity, and that the experimental protocol followed does not?	√	
2.8 Are details of media preparation (**type of media, serum,** any **added antibiotics**) provided?	√	
2.9 Is a **justification of the biological model** used provided? For examples for cancer models, see *Cancer Res.* **75** (2015) 4016; http://doi.org/10.1158/0008-5472.CAN-15-1558, and *Mol. Ther*. **20** (2012) 882; http://doi.org/10.1038/mt.2012.73, and *ACS Nano* **11** (2017) 9594; http://doi.org/10.1021/acsnano.7b04855	not applicable	
2.10 Is characterization of the **biological fluid** (*ex vivo*/*in vitro*) reported? For example, when investigating protein adsorption onto nanoparticles dispersed in blood serum, pertinent aspects of the blood serum should be characterised (e.g., protein concentrations and differences between donors used in study).	not applicable	
2.11 For **animal experiments**, are the ARRIVE guidelines followed? For details, see *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412	not applicable	
Explanation for **No** (if needed): 2.4: Cells were purchased from ATCC. The passage number was reported and regular mycoplasma test was conducted.		

*For *in vitro* experiments (e.g., cell culture), *ex vivo* experiments (e.g., in blood samples), and *in vivo* experiments (e.g., animal models). The questions above that are appropriate depend on the type of experiment conducted.

Supplementary Table 3. Experimental details*

Question	Yes	No
3.1 For cell culture experiments: are **cell culture dimensions** including **type of well**, **volume of added media**, reported? Are cell types (i.e.; adherent vs suspension) and **orientation** (if non-standard) reported?	√	
3.2 Is the **dose of material administered** reported? This is typically provided in nanomaterial mass, volume, number, or surface area added. Is sufficient information reported so that regardless of which one is provided, the other dosage metrics can be calculated (i.e. using the dimensions and density of the nanomaterial)?	√	
3.3 For each type of imaging performed, are details of how **imaging** was performed provided, including details of **shielding, non-uniform image processing**, and any **contrast agents** added?	√	
3.4 Are details of how the dose was administered provided, including **method of administration, injection location, rate of administration,** and details of **multiple injections**?	not applicable	
3.5 Is the methodology used to **equalise dosage** provided?	√	
3.6 Is the **delivered dose** to tissues and/or organs (in vivo) reported, as % injected dose per gram of tissue (%ID g^{-1})?	not applicable	
3.7 Is **mass of each organ/tissue measured** and **mass of material** reported?	not applicable	
3.8 Are the **signals of cells/tissues with nanomaterials** reported? For instance, for fluorescently labelled nanoparticles, the total number of particles per cell or the fluorescence intensity of particles + cells, at each assessed timepoint.	√	
3.9 Are **data analysis details**, including **code used** for analysis provided?	√	
3.10 Is the **raw data** or **distribution of values** underlying the reported results provided? For examples, see *R. Soc. Open Sci.* **3** (2016) 150547; http://doi.org/10.1098/rsos.150547, https://opennessinitiative.org/making-your-data-public/, http://journals.plos.org/plosone/s/data-availability, and https://www.nature.com/sdata/policies/repositories	not applicable	
Explanation for **No** (if needed):		

* The use of protocol repositories (e.g., *Protocol Exchange* http://www.nature.com/protocolexchange/) and published standard methods and protocols (e.g., *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235, and *Chem. Mater.* **29** (2017) 475; http://doi.org/10.1021/acs.chemmater.6b05481) are encouraged.