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Supporting Text

Materials. Chemicals used for synthesis of monomers and polymers were obtained from Aldrich unless otherwise indicated. Tetrahydrofuran was distilled under a nitrogen atmosphere from Na/benzophenone immediately before use. *p*-Toluenesulfonic acid was dehydrated by heating at 100ºC under vacuum for 6 h and then recrystallized from boiling toluene. *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide and acryloyl chloride were freshly distilled before each use. Merck Kieselgel plates coated with silica gel 60 F_{254} were used for TLC and were visualized by UV activity (λ = 254 nm). Flash column chromatography was done with Merck Kieselgel 60 silica gel (230 μm).

Characterization. ^{1}H NMR spectra were recorded at 300 or 400 MHz, and ^{13}C spectra were recorded at 100 MHz. NMR chemical shifts are reported in ppm relative to tetremethylsilane and are calibrated against residual solvent peaks: $CDCl_3$ (δ 7.26, δ 77.23) or DMSO-d_6 (δ 2.49, δ 39.51). All coupling constants are reported in Hz. High-resolution fast atom bombardment MS (FAB-HRMS) experiments were performed at the University of California at Berkeley MS facility. Fourier transform IR spectroscopic analyses were performed by using a thin film cast from $CHCl_3$ on a reflective mirror surface. Elemental analyses were performed by M-H-W Laboratories or the University of California at Berkeley Analytical Laboratory.

1-Chloro-3,6,9-trioxadecane (3). This compound was prepared according to the procedure reported by Loth and Ulrich (1). Spectroscopic data agreed with those reported in the literature. ^{13}C NMR($CDCl_3$): δ 42.16, 58.30, 69.92, 69.96, 69.98, 70.70, 71.30. Analytically calculated for $C_7H_{15}O_3Cl$: C, 46.03; H, 8.28. Found: C, 45.68; H, 8.39, yield 70%.

***p*-(1,4,7,10-Tetraoxaundecyl)benzaldehyde (4).** Chloride **3** (26 g, 0.14 mol, 1.3 equiv) and *p*-hydroxybenzaldehyde (13 g, 0.11 mol, 1 equiv) were dissolved in dry tetrahydrofuran (40 ml). K_2CO_3 (15 g, 0.11 mol, 1 equiv) was added followed by 18-crown-6 (3.0 g, 11 mmol, 0.11 equiv) and KI (0.20 g, 1.2 mmol, 0.01 equiv). The reaction mixture was stirred at reflux for 48 h. The resulting mixture was cooled to room temperature, and water (200 ml) was added. The product was extracted with 3 × 350 ml portions of ethyl acetate and the combined organic layers were dried and concentrated. The oil was loaded onto a silica gel column and eluted with a 1:9 mixture of ethyl acetate/hexane, followed by a ratio of 1:4, 3:7, 4:1 of ethyl acetate/hexane, and finally washed with ethyl acetate to afford 21 g (70%) of **4** as a clear oil. IR(cm^{-1}): 1697 (s), 1165 (s). ^{1}H NMR (400, $CDCl_3$): δ 3.36 (s, 3), 3.54 (t, 2, J = 4.6), 3.63-3.68 (m, 4), 3.72-3.75 (m, 2), 3.88 (t, 2, J = 4.8), 4.20 (t, 2, J = 4.8), 7.01 (d, 2, J = 8.8), 7.82 (d, 2, J = 8.8), 9.87 (s, 1). ^{13}C NMR ($CDCl_3$): δ 58.86, 67.59, 69.29, 70.40, 70.47, 70.71, 71.73, 114.71, 129.84, 131.76, 163.69, 190.63. Calculated: $[M+H]^+$ ($C_{14}H_{21}O_5$) *m/z* = 269.13889. Found FAB-HRMS: $[M+H]^+$ *m/z* = 269.134487. Analytically. calculated for $C_{14}H_{20}O_5$: C, 62.67; H, 7.51. Found: C, 62.47; H, 7.74.

***N,N*'-Bistrifluoroacetyl-di-(2-aminoethoxy)-[4-(1,4,7,10-tetraoxaundecyl)phenyl]methane (5).** Aldehyde **4** (3.60 g, 13.4 mmol, 1 equiv) and *N*-(2-hydroxyethyl)-2,2,2-trifluoroacetamide (15.0 g, 95.5 mmol, 7.1 equiv) were dissolved in dry tetrahydrofuran (50 ml). *p*-Toluenesulfonic acid (0.360 g, 2.09 mmol, 0.16 equiv) and 5 Å molecular sieves (50 g) were added. The reaction mixture was stirred overnight and quenched with triethylamine (10 ml, 72 mmol, 5.3 equiv). The reaction mixture was filtered to remove the molecular sieves with a buchner funnel. A 150 ml portion of water was added to the filtrate and then extracted with four 150-ml portions of ethyl acetate. The ethyl acetate was evaporated and the product was recrystallized twice from ethyl acetate/hexane, recovering 4.03 g of **5** (60%). Melting point: 90.2-91.3 °C. IR (cm^{-1}): 3280 (br), 1702 (s), 1562 (m), 1210 (s), 1180 (s). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.22 (s, 3), 3.37-3.42 (m, 6), 3.49-3.57 (m, 10), 3.72 (t, 2, J = 4.6), 4.07 (t, 2, J = 4.6), 5.52 (s, 1), 6.91 (d, 2, J = 8.7), 7.29 (d, 2, J = 8.7), 9.51 (t, 2, J = 5.5). ^{13}C NMR (DMSO-d_6): δ 39.22, 58.00, 62.57, 67.12, 68.91, 69.59, 69.78, 69.93, 71.26, 100.57, 113.93, 116.14 (q, J

= 241), 127.72, 130.26, 156.40 (q, J = 36), 158.52. Calculated: $[M]^+$ ($C_{22}H_{30}F_6N_2O_8$) *m/z* = 564.1906. Found FAB-HRMS: $[M]^+$ *m/z* = 564.1922. Analytically calculated for $C_{22}H_{30}F_6N_2O_8$: C, 46.81; H, 5.36; N, 4.96. Found: C, 46.97; H, 5.38; N, 4.72.

***N,N*'-Bisacryloyl-di-(2-aminoethoxy)-[4-(1,4,7,10-tetraoxaundecyl)phenyl]methane (6).** Compound **5** (4.0 g, 7.1 mmol, 1 equiv) and 6 M NaOH (30 ml) were added to dioxane (20 ml) and the reaction mixture was stirred at room temperature for 7 h. Complete removal of the acetamide groups was determined by TLC using ninhydrin staining. Upon completion, the reaction mixture was cooled to 0°C and triethylamine (3 ml) was added. Acryloyl chloride (12 ml, 0.15 mol, 21 equiv) and triethylamine (36 ml, 0.26 mol, 36 equiv) were added in small alternating portions while periodically monitoring the pH to maintain it above 7. A 10% K_2CO_3 in water solution (30 ml) was added, and the reaction mixture was stirred for 10 min before extracting the product with four 200-ml portions of ethyl acetate. The organic layers were combined, dried, and evaporated and the product was crystallized from ethyl acetate/hexane, yielding 2.05 g (58%) of **6** as a white solid. Melting point: 83.6-85.5 °C. IR (cm^{-1}): 3293 (br), 1665 (s), 1562 (s), 1245 (s), 1101 (s). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.38 (s, 3), 3.53-3.76 (m, 16), 3.86 (t, 2, J = 4.8), 4.14 (t, 2, J = 4.8), 5.44 (s, 1), 5.65 (dd, 2, J = 17, J = 2), 6.15 (dd, 2, J = 17, J = 10), 6.22 (s, 2), 6.30 (dd, 2, J = 10, J = 2), 6.91 (d, 2, J = 8.8), 7.32 (d, 2, J = 8.8). ^{13}C NMR (DMSO-d_6): δ 38.71, 58.04, 63.80, 67.10, 68.93, 69.61, 69.80, 69.94, 71.27, 100.83, 113.91, 125.10, 127.86, 130.62, 131.69, 158.45, 164.72. Calculated: $[M+H]^+$ ($C_{24}H_{37}N_2O_8$) *m/z* = 481.2549. Found FAB-HRMS: $[M+H]^+$ *m/z* = 481.2544. Analytically calculated. for $C_{24}H_{36}N_2O_8$: C, 59.99; H, 7.55; N, 5.83. Found: C, 59.86; H, 7.75; N, 5.77.

Protein Encapsulation Measurements. Two milligrams of each microgel sample (see Table 1), containing Cascade Blue-labeled ovalbumin, was dispersed in 0.5 ml of 300 mM sodium phosphate-buffered water at pH 8.0 by sonication for 5 min. The microgel samples were centrifuged for 5 min and the supernatant was pipetted off to remove any unbound protein. The washed microgels were then hydrolyzed in 300 mM sodium acetate buffered water (pH 1.6, 500 μl). After complete hydrolysis of the microgels, the quantity

of encapsulated protein was determined by fluorescence spectroscopy, excitation at 405 nm, emission at 460 nm. The protein concentration of each microparticle sample was calculated by fitting the emission to a calibration curve made from known concentrations of Cascade Blue-labeled ovalbumin.

Protein Release Measurements. Two milligrams of each microgel sample (see Table 1), containing Cascade blue-labeled ovalbumin, was dispersed in 0.5 ml of 300 mM sodium phosphate buffered water at pH 8.0 by sonication for 5 min. The microgel samples were centrifuged for 5 min and the supernatant was pipetted off to remove any unbound protein. The recovered pellet was then redispersed into either 300 mM acetic acid buffered water (pH 5.0, 500 μl) or 300 mM sodium phosphate buffer (pH 7.4, 500 μl). The solutions were incubated at 37°C in a heating block for each time point. The percentage of protein released at a given time point was determined by centrifuging the microparticle sample for 5 min, isolating the supernatant from the pellet, and comparing the fluorescence of the supernatant (released protein) with that of the pellet (protein still in beads), excitation at 405 nm, emission at 460 nm. The recovered pellet was hydrolyzed in 300 mM acetic acid at pH 1.6, before measuring its fluorescence. The background emission of each buffer was measured and subtracted from all of the readings.

Toxicity of Microgels Made with 6. The toxicity of ovalbumin-loaded microgels was measured with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay by using RAW 309.CR1 cells. RAW 309.CR1 cells were split at 5×10^4 cells per well in a 96-well plate and allowed to grow overnight. The cells were then incubated with the microgels (1.6% crosslinked, sample A from Table 1) for 16 h in DMEM with 10% FBS. The microgels were aspirated from the cells, and they were then washed several times with PBS and allowed to grow for another 48 h. The cell viability was determined with the MTT reagent by using the protocol described in Freshney (2).

1. Loth, H. & Ulrich, F. (1998) *J. Controlled Release* **54,** 273–282.

2. Freshney, I. R. (1994) *Culture of Animal Cells* (Wiley-Liss, New York).