Miktoarm Stars of Poly(ethylene oxide) and Poly(dimethylaminoethyl methacrylate): Manipulation of Miscellation by Temperature and Light

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Experimental

Materials

Poly(ethylene glycol) monomethyl ether (Mn = 5000 g/mol; PDI = 1.1) was delivered by Polysciences, Warrington, PA, USA. N,N-dimethylaminoethyl methacrylate (DMAEMA; purified by filtration over basic alumina) and molecular sieves (3 Å, 8 to 12 mesh) was purchased from Acros Organics, Geel, Belgium. Methanesulfonyl chloride was delivered by Riedel de-Haën, Germany. Dichloromethane, chloroform, tetrahydrofuran (THF; distilled over sodium / benzophenone), diethylether, methanol and acetone were bought from J.T. Baker (Holland). Acetic acid was purchased from AppliChem (Darmstadt, Germany). Hydrochloric acid was obtained from FF-Chemicals Oy (Finland) or Merck (Germany). Poly(ethylene glycol) (10 g; 1.9 × 10^{-3} mol) was dissolved in dichloromethane (20 ml dry THF under nitrogen-countercflow at RT). After 1.5 h of stirring, the PEO solution was slowly (over 10 min) added with a syringe under nitrogen counterflow. Then the precipitate was washed with n-hexane before the powder was dried in desiccator under vacuum (25 g, 50%). SEC (THF; PEO calibration): Mn = 4600 g/mol, PDI = 1.1; δ(200 MHz; CDCl3; MeSi) 4.38 (2H, m), 3.98 (2H, m), 3.8-3.5 (m, PEO-H), 3.38 (3H, s, methoxy), 3.28 (2H, m), 3.09 (3H, s, mesylate); δ(50.3 MHz; CDCl3; MeSi) 72.1, 70.9 (PEO), 69.6, 69.2, 59.2 (CH3-O-), 37.9 (CH3-SO3-)

Synthesis

Monomesylated poly(ethylene glycol) CH3O-PEO114-Mes [monomethyl poly(ethylene oxide) methanesulfonate]. Poly(ethylene glycol) monomethyl ether (50 g; 0.01 mol) was dissolved in 160 g dichloromethane and triethylamine (10 g; 0.1 mol), before 1 g molecular sieves were added under stirring. After 1 h the sieves were removed and the mixture was cooled under stirring to 0°C. Then methanesulfonyl chloride (2 g; 0.017 mol) was added dropwise under nitrogen counterflow and the mixture was slowly allowed to warm up to RT for 16 hours. Then the mixture was concentrated in vacuo and dissolved in 200 mL dichloromethane in order to filtrate the orange mixture through silica. Then the filtrate was precipitated in 800 mL anhydrous diethylether, separated from the supernatant and again dissolved in 200 mL dichloromethane to repeat the filtration and precipitation procedure. Then the precipitate was washed with n-hexane before the powder was dried in desiccator under vacuum (25 g, 50%). SEC (THF; PEO calibration): Mn = 4600 g/mol, PDI = 1.1; δ(200 MHz; CDCl3; MeSi) 4.38 (2H, m), 3.98 (2H, m), 3.8-3.5 (m, PEO-H), 3.38 (3H, s, methoxy), 3.28 (2H, m), 3.09 (3H, s, mesylate); δ(50.3 MHz; CDCl3; MeSi) 72.1, 70.9 (PEO), 69.6, 69.2, 59.2 (CH3-O-), 37.9 (CH3-SO3-)
Each fraction was checked by SEC and finally all good fractions were combined to give the final product (0.5 g, 5%). SEC (THF; PEO calibration): $M_n = 8400$ g/mol, $PDI = 1.20$; $\delta n(200$ MHz; CDCI$_3$; MeSi$) = 3.98$ (4H, m, PEO), $3.8-3.5$ (m, PEO-CH$_2$-O-CH$_2$-O-CH$_2$-O-), $3.28$ (4H, m, PEO); $\delta n_{50.3$ MHz; CDCI$_3$; MeSi$) = 72.2$ (broad), 70.8 (predominantly PEO), 69.6, 68.7 (broad), 65.6 (-CH$_2$-O-), 59.3 (CH$_3$-O-), 45.7 (quaternary C)

Macroinitiator with four ATRP initiation sites PEO$_{114}$-Br$_6$-PEO$_{114}$ (poly(ethylene oxide) - block - 2,2,6,6-tetramethylcyclohexanecarbodiimide)-4-oxa-1,7-heptandiol - block - poly(ethylene oxide)). Diblock PEO with inner dipentaerythritol moiety (0.12 g; $4.8 \times 10^{-5}$ mol OH-Groups) was dissolved in 1 mL dry THF and nitrogen. Additionally 10 beads of molecular sieves (DMF, 1 g/L LiBr; PEO calibration): $M_n = 8100$ g/mol, $PDI = 1.18$; $\delta n(200$ MHz; CDCI$_3$; MeSi$) = 4.22$ (8H, s, dipentaerythritol -CH$_2$-O-C=O), 3.98 (4H, m, PEO), $3.8-3.5$ (m, PEO-CH$_2$-O-CH$_2$-O-CH$_2$-O-), $3.28$ (4H, m, PEO), 1.93 (24H, s, O-C-C(CH$_3$)$_2$Br); $\delta n_{50.3$ MHz; CDCI$_3$; MeSi$) = 70.8$ (weak signal, quaternary C, -CH$_2$-O-), 72.2, 70.8 (predominantly PEO), 69.6, 68.7 (broad), 64.0 (-CH$_2$-O-), 45.7 (quaternary C, dipentaerythritol), 31.2 (O-C-C(CH$_3$)$_2$Br)

Macroinitiator with four ATRP initiation sites PEO$_{114}$-Br$_6$-PEO$_{114}$ (poly(ethylene oxide) - block - 2,2,6,6-tetramethylcyclohexanecarbodiimide)-4-oxa-1,7-heptandiol - block - poly(ethylene oxide)). Diblock PEO with inner dipentaerythritol moiety (0.13 g; $4.8 \times 10^{-5}$ mol OH-Groups) was dissolved in 1 mL dry THF and nitrogen. Additionally 10 beads of molecular sieves were added and the mixture was allowed to dry for 30 min. 2,2-bis(2-bromoisobutyroyl-oxymethyl) propionic acid (77 mg; $1.8 \times 10^{-4}$ mol), dicyclohexylcarbodiimide (DCC, 60 mg; $2.9 \times 10^{-4}$ mol) and p-N,N-dimethylaninopyridin (DMAP, 0.5 mg) were added and stirred for another day. Then further 30 mg of initiator fragment in 1 mL THF was added and stirred for another 3 days. Then the mixture was diluted with 2 mL THF and filtrated. The THF solution was then dialyzed against THF for 6 days (MWCO 6000 – 8000) and then the dialysis was continued against water for 4 h, filtrated with syringe filter and freeze dried to obtain 100 mg of macroinitiator (100 mg, 80 %; $^1$H NMR shows 70% esterification conversion - macroinitiator bears on average 6 initiation sites). $\delta n(200$ MHz; CDCI$_3$; MeSi$) = 4.39$ (11H, m, -CH$_2$-O-C=O,-C(CH$_3$)$_2$Br), 3.98 (4H, m, PEO), $3.8-3.5$ (m, PEO-CH$_2$-O-CH$_2$-O-), 3.29 (4H, m, PEO), 1.92 (33H, s, O-C-C(CH$_3$)$_2$Br); 1.33 (8H, s, CH$_2$-C(CH$_3$-O-)$_2$C(=O)) (DMF, 1 g/L LiBr; PEO calibration): $M_n = 8100$ g/mol, $PDI = 1.20$; $\delta n(200$ MHz; CDCI$_3$; MeSi$) = 3.98$ (4H, m, PEO), $3.8-3.5$ (m, PEO-CH$_2$-O-CH$_2$-O-), $3.28$ (4H, m, PEO); $\delta n_{50.3$ MHz; CDCI$_3$; MeSi$) = 70.8$ (predominantly PEO), 69.6, 68.7 (broad), 65.6 (-CH$_2$-O-), 59.3 (CH$_3$-O-), 45.7 (quaternary C)

Miktoarm star with 2 PEO arms and 4 PDMAEMA arms (PEO$_{114}$-(PDMAEMA$_{40}$)$_2$-PEO$_{114}$). The macroinitiator PEO$_{114}$-Br$_4$-PEO$_{114}$ (40 mg; $M_n \sim 10800$ g/mol; $1.48 \times 10^{-5}$ mol Br groups), copper(I)chloride (CuCl; 1 g/L; 11 mg; $1.1 \times 10^{-5}$ mol) and copper(II)chloride (CuCl$_2$; 0.5 mg; $3.8 \times 10^{-6}$ mol) were mixed in anisole (0.5 g) and deoxygenated by purging with nitrogen. Then the ligand $N,N,N,N'-N''-N'''-N''''-N''''''$hexamethyldiethyramine (HMTETA; 33 mg; $1.4 \times 10^{-4}$ mol) and the monomer $N,N$-dimethylaminoethoxy methacrylate (PDMAEMA; 233 g; $1.48 \times 10^{-2}$ mol) were also mixed and deoxygenated. 0.254 g of this solution (0.250 g DMAEMA - 1.59 $10^{-4}$ mol; 3.5 mg HMTETA - 1.52 $10^{-5}$ mol was introduced to the macroinitiator mixture at 80°C under stirring and nitrogen counterflow. After 190 min the reaction was terminated by injection of tributyltinhydride (10 mg; $3.4 \times 10^{-5}$ mol; mixture turns brownish-black). 2 Reaction was stopped after one additional hour at 80°C by dilution with chloroform (5 mL) and contact with air. The conversion was 37 % according to NMR ($P_{n,th()}(arm) = 40$; $M_{n,th()}(total) = 35800$ g/mol). Then the polymer solution was filtrated through silica and then reprecipitated before it was precipitated from n-hexane. Then the precipitate was dissolved in 4 mL THF and dialyzed against THF for 1 day (MWCO 12000 - 14000) to yield after drying in vacuo 80 mg of miktoarm star. SEC (DMF, 1 g/L LiBr; PEO calibration): $M_{n,app} = 10600$ g/mol, $PDI_{app} = 1.25$; $M_{n,osmometry} = 44000$ g/mol; $\delta n(200$ MHz; CDCI$_3$; MeSi$) = 4.2 - 3.9$ (O-CH$_2$-CH$_2$-N), 3.8 - 3.5 (PEO-CH$_2$-N), 2.7-2.4 (O-CH$_2$-CH$_2$-N), 2.4 - 2.1 (-N(CH$_3$)$_2$), 2.1 - 1.6 (PDMAEMA backbone CH$_2$); 1.2 - 0.7 (PDMAEMA backbone CH$_3$)

Cleaving of the arms. 5 mg of polymer PEO$_{114}$-Br$_4$-PEO$_{114}$ (PDMAEMA$_{40}$)$_2$-PEO$_{114}$ was retained from solutions used for light scattering by repeated dialyses from salted solutions and final freeze drying. The polymer was dispersed in 0.5 mL concentrated NaOH and kept at 85 °C for 3 days. Then the solution was acidified by addition of 1 mL of concentrated HCl. Again the solution was kept at 85 °C for 3 days. Then the pH of the solution was set to pH 14 by careful addition of solid NaOH. This solution was kept at 85 °C for 3 days before the cooled solution (diluted to 5 mL) was extracted three times with 5 mL chloroform each. The aqueous phase was again kept for 1 day at 85 °C, before its pH was adjusted to pH 3 for HCl and then freeze dried. The dried salts were
dissolved in 3 mL water and were dialyzed against water (MWCO 1000) and after 1 day the dialysis tubing was opened to add 0.1 mL 1 M HCl. The dialysis was continued against pure water for 3 days before the solution was finally freeze dried (to get ~ 2 mg of PMMA). Then the dried poly(methacrylic acid) PMAA was dissolved in 0.2 mL water and 2 mL THF before 0.1 mL of 2 M trimethylsilyldiazomethane in diethyl ether was added. After one hour stirring at RT, the solution was dialyzed against THF (MWCO 1000) for 1 day, before the solution was dried to obtain 2 mg of PMMA).

Synthesis of miktoarm star with 2 PEO arms and 3 PDMAEMA arms (PEO_{114}-Br, PEO_{114} (40.46 mg; M_n~11500 g/mol; M_w~19000 g/mol; 2.11 × 10^{-5} mol Br groups), copper(II)chloride (CuCl; 1.66 mg; 1.68 × 10^{-2} mol), copper(I)chloride (CuCl_2; 0.56 mg; 4.2 × 10^{-6} mol) and anisole (0.72 g) were mixed in a flask, equipped with septum and stirrer. The mixture was deoxygenated by purging with nitrogen. N,N,N’,N”,N”’,N”’-hexamethyldiethytriamine (HMETA; 19.2 mg; 8.3 × 10^{-5} mol) was dissolved in N,N-dimethylformamide (DMAEMA; 1.32 g; 8.4 × 10^{-3} mol) and also deoxygenated by purging with nitrogen. Then 0.330 g of the monomer mixture (0.325 g DMAEMA - 2.09 × 10^{-2} mol; 4.7 mg HMETA - 2.0 × 10^{-5} mol) was introduced to the macroinitiator mixture under nitrogen counterflow at 80 °C. After 60 min the polymerization was stopped by dilution with chloroform (5 mL), contact with air and cooling with ice water. The conversion was 25% according to NMR (P_n,th eo (arm) = 26). Then the polymer solution was filtrated over silica and then recondensated before it was precipitated from hexane. Then the precipitate was taken up in 4 mL THF and dialyzed against THF for 1 day (MWCO 12000 - 14000) to yield after drying in vacuo 110 mg of Polymer. SEC (DMF, 1 g/L LiBr; PEO calibration): M_n,app = 9500 g/mol, PDI_{app} = 1.23; M_n(osmometry) = 31000 g/mol; δm(200 MHz; CDCl_3; Me_4Si) 4.3 - 3.9 (O-CH_2-CH_2-N), 3.8 - 3.5 (PEO-H), 2.7-2.4 (O-CH_2-CH_2-N), 2.4 - 2.1 (-N(CH_3)_2), 2.1 - 1.6 (PDMAEMA backbone CH_3); 1.2 - 0.7 (PDMAEMA backbone CH_3).

Quaternization. 10 mg of polymer PEO_{114}-Br, PEO_{114} was dissolved in 5 mL of THF and 1 mL of acetonitrile before 0.1 mL of methyliodide was added. The solution was stirred light protected over night at RT. Then the solution was dialyzed against THF for 24 h (MWCO 6000 - 8000) and then against water for another 24 h before the solution was freeze dried (15 mg product).

Cleaving of the arms. 10 mg of quaternized miktoarmstar PEO_{114}-Br (PDMAEMA_{55})_3-PEO_{114} dissolved in 1 mL of concentrated aqueous NaOH solution and kept at 85 °C for 5 days. The cooled solution was carefully brought to pH 3 with concentrated HCl and freeze dried. Then the residue was dialyzed against water for 1 day (MWCO 1000) and freeze dried in order to extract the dry residue with 10 mL methanol (containing 1 g/L NaOH) for 1 day at RT. The supernatant methanol was then rejected, whereas the residue was seen by 1H-NMR as almost pure sodium salt of poly(methacrylic acid) PMAA. The PMAA was therefore dissolved in 2 mL pH 3 HCl and dialyzed for 1 day before 1 mL of 0.1 M HCl was added into the dialysis tube. The dialysis was continued for 6 h against water (MWCO 1000), before the mixture was finally freeze dried. Then the dried poly(methacrylic acid) PMAA was dissolved in 0.2 mL water and 2 mL THF before 0.1 mL of 2 M trimethylsilyldiazomethane in diethyl ether was added. After one hour stirring at RT the solution was dialyzed against THF (MWCO 1000) for 1 day and then dried (5 mg of PMMA).

Polymer Characterization

NMR Spectroscopy. The NMR spectra were measured with a 200-MHz Varian Gemini 2000 NMR spectrometer (operating at 200 MHz for 1H and at 50.3 MHz for 13C) using CDCl_3 as solvent (usually 10 mg/mL). The chemical shifts are presented in parts per million downfield from the internal TMS standard. Simulations were performed with ACD/HNMR and ACD/CNMR Predictor Ver.3.00.

Size Exclusion Chromatography (SEC). The SEC analyses were performed with a Waters instrument equipped with a Styragel guard column, 7.8 x 300 mm Styragel capillary column, and Waters 2487 UV and Waters 2410 RI detectors. Dimethylformamide with 1 g/L LiBr was used as an eluent at a flow rate of 0.8 mL/min (in some cases THF, where noted). PEO samples were carefully heated with a fan to accelerate the dissolution process. The conventional calibrations was performed with poly(ethylene oxide) (for samples containing PEO; Polymer Laboratories, Amherst, USA) or poly(methyl methacrylate) standards (for PMMA samples; Polymer Standards Service PSS, Mainz, Germany).

Matrix Assisted Laser Desorption Ionization – Time of Flight (MALDI-ToF) Mass Spectrometry. MALDI-ToF mass spectrometry was performed on a Bruker microflex equipped with 337 nm N_2 laser in the linear mode (accelerating voltage 20 kV, pressure 5 × 10^{-6} mbar) for determination of molecular weight of miktoarm stars and cleaved off and modified PDMAEMA arms (PMMA). THF solutions of dithranol (20 µL of 20 g/L), sodium trifluoroacetate (0.5 µL of 10 g/L) and analyte (5 µL of 10 g/L PMMA) were mixed and 0.5 µL were applied on sample plate. For miktoarm stars α- cyano-4-hydroxycinnamic acid (CHCA) was used as matrix (45 µL of 10 g/L in DMSO) mixed with polymer solution in THF (5 µL of 10 g/L). This mixture was applied on a sample plate (1 µL). In case of overlapping peaks (e.g. double charged species / matrix related signals) Gaussian curve fitting was performed in order to calculate number average molar mass and polydispersity index.

Osmometry. A membrane osmometer (Osmomat 090, Gonotec GmbH, Berlin, Germany) with regenerated cellulose membrane (Gonotec two layer membrane 90.9.010; cut off 20000 dalton) was used for the determination of the molecular weight of miktoarm stars. Solutions with different
concentrations in THF were injected to extrapolate to zero concentration. The cell was kept at 30 °C. To rinse the measurement cell with a new sample, approximately 0.7 mL of sample solution were injected three times.

**Sample Preparation.** The polymer concentrations were usually 0.12 g/L or below. 0.42 mg of miktoarm was dissolved in 3 mL of pH 8 buffer, containing additional 0.1 M NaCl. Then 0.54 mL of 0.0166 M K3[Co(CN)6] were added, keeping the ionic strength basically constant (yielding 2.5 × 10^{-3} M [Co(CN)6]3-). For diluted samples, a 0.12 g/L solution was diluted with just solvent of pH 8 buffer, 0.1 M NaCl and 2.5 mM [Co(CN)6]3-

**Light Scattering.** Some temperature-dependent static light scattering (SLS) and dynamic light scattering (DLS) measurements were obtained using Malvern Nano ZS instrument equipped with red laser (λ = 633 nm) and using backscattering optics (equilibration time 10 min between different temperatures; the measurements were performed with increasing temperature from 5 °C to 80 °C with 2.5 K intervals after first cooling to 5 °C with ~ 0.3 K/min). DLS and SLS measurements were otherwise conducted with Brookhaven Instruments BI-9000AT digital correlator and BI-200SM goniometer, which was equipped with a thermostat. Diode laser Mini-L30 (λ = 637.6 nm; 30 mW; Brookhaven Instruments) was used as a light source. The SLS data were analyzed by using relative scattered intensities compared to the scattering of toluene. In order to determine the molar mass of aggregates at 10 °C, we used Zimm’s double extrapolation method. In order to determine the molar mass of aggregates at 10 °C, we used Zimm’s double extrapolation method. For PEO114-(PDMAEMA55)-PEO114 each solution was first heated to 30 °C and cooled to 10 °C with 20 K/h to guarantee similar aggregation numbers and sizes for all solutions. The specific refractive index increments of the polymers (dn/dc) were determined from refractive indices measured by a Billingham & Stanley Abbe60 refractometer using the same light source as for the scattering experiments. E.g. 32 g/L solution of PEO114-(PDMAEMA55)-PEO114 in 0.1 M NaCl, pH 8 buffer and 2.5 mmol/L [Co(CN)6]3- was dialyzed against same solvent and dialyzed with collimating adaptor (EXFO 810-00042; S/N:0067), kept at 9 cm distance from sample (0.4 mW/cm²).

**Cryo Transmission Electron Microscopy (cryo-TEM).** The samples of PEO114-(PDMAEMA40)-PEO114 were prepared by dissolving 0.42 mg of miktoarm star in 3 mL of buffered 1 M NaCl (pH 8) and 0.54 mL of 0.0166 M K3[Co(CN)6]. A drop of the sample (3 µL) was put on carbon coated copper grid R 2/2 (Quantifoil Micro Tools GmbH, Jena, Germany; hydrophilized by glow discharge unit Emitech KX100, 25 mA/min). The drop was kept at respective temperature (5 or 40 °C using Fei Vitrobot; 100 % humidity) for 15 min. For vitrification from 70 °C the sample was thermostated for 15 min in 70 °C water bath before applying one drop into the Vitrobot, which was kept at 60°C, instantly allowing the vitrification. Vitrification was performed by using a plot time of 1.5 or 3 s, zero off-set, no drain time, and liquid ethanol / propane mixture (1:1 volume) as a coolant (cooled below -180 °C). PEO114-(PDMAEMA55)-PEO114 samples were cooled down to 5 °C with 20 K/h and then applied into the thermostated Vitrobot (5 °C). Samples were maintained at -184 °C in a Gatan 910 cryoholder whilst images were recorded on a FEI Tecnai 12 transmission electron microscope operated at 120 kV under the low-dose conditions. All images were registered digitally by a bottom-mounted CCD camera system (Ultrascan 1000, Gatan) and processed with a digital imaging processing system (Gatan Digital Micrograph).

**Steady-state Fluorescence Spectroscopy.** Fluorescence spectroscopy of miktoarm dissolved in 0.1 M NaCl, pH8 buffer, 2.5 10^{-3} M K3[Co(CN)6] was dissolved to 2 mL of sample (e.g. 0.12 g/L miktoarm dissolved in 0.1 M NaCl, pH8 buffer, 2.5 10^{-3} M K3[Co(CN)6]; other concentrations of polymer were achieved by dilution with saline solvent). Those solutions were kept over night at 4 °C for equilibration. Fluorescence spectra were recorded using PTI Photon Technology International spectrophotometer (right-angle geometry, PTI 814 Photomultiplier Detection System; lamp unit PTI LPS-220B; ByteBox interface; FeliX32 processing software; half micro quartz sample cell QS, 10.00 mm, 0.8 mL; University of Helsinki, Center for Drug Research) using the following conditions: excitation at 470 nm, recording range 500-680 nm, slit width 4 nm for excitation and emission. The spectrometer was equipped with a thermostat.

**Irradiations.** UV-lamp Omnirıce Series 1000 (100 W; University of Helsinki, Center for Drug Research), equipped with 365 nm filter (output between 325 nm and 410 nm with maximum at 365 nm) was used for UV-illuminations. Illuminations were performed at 50 % opened iris. The light was directed to the sample by use of a light guide, equipped with collimating adaptor (EXFO 810-00042; S/N:0067), kept at 9 cm distance from sample (0.4 mW/cm²).

**Additional Comments to Synthesis**

Two polymerizations of DMAEMA were conducted with two different macrorinitiators, yielding allmost the same molecular weigh for both miktoarm stars. One pathway
headed for stars with up to 8 PDMAEMA arms, the other limited the PDMAEMA arm number to 4. Therefore the initiator fragment depicted in Scheme S1 was applied in addition to the initiator shown in Scheme 1 (main part of publication). The polymerization employing the initiator with 4 initiation sites (Scheme 1) was stopped with tributyltin hydride in order to replace the halogen with hydrogen. This exchange prevents the possible substitution of the endgroup halogen with amino groups, causing intra- or intermolecular cross linking of polymers. Since in the course of this exchange the conversion in monomer consumption is not totally controlled, the second polymerization with higher number of initiation sites per molecule (Scheme S1) was stopped just by cooling, dilution with chloroform and with contact with air. The 1H-NMR spectra of the intermediates for the preparation of miktoarm stars with up to 4 PDMAEMA arms are depicted in Figure S1.
Fig. S2. MALDI-ToF mass spectra of miktoarm stars (left hand side) and their cleaved off arms (after transformation to PMMA, right hand side); top figures relate to PEO114-(PDMAEMA40)4-PEO4, whereas the bottom row originates from PEO114-(PDMAEMA55)3-PEO114 (red curves are Gaussian fits, partly extracted by double Gaussian fitting).

However the cleavage of arms revealed, that especially the growth of arms from the dendritic initiation fragments is hindered probably due to increasing sterical demands. In order to determine the number of arms, we either prehydrolyzed the polymer by a treatment with hydrochloric acid or we quaternized the amino groups of the polymer. Both procedures help to increase the solubility of the polymer in basic water. Then all the ester bonds were cleaved by alkaline treatment. The cleaved PEO was removed by extraction with alkaline MeOH (PMAA hardly dissolves) or by extraction with CHCl3. The alkaline treatment works best for longer PMAA. The resulting poly(methacrylic acid) PMAA was methylated to obtain poly(methyl methacrylate) PMMA, which was analyzed by MALDI-ToF mass spectrometry. The final MALDI-ToF mass spectra of cleaved off arms are depicted in Figure S2 (together with mass spectra of the whole miktoarm stars). The peaks were fitted by Gaussian functions, since always additional peaks from matrix or double charged species could be observed. For the smallest molecular weight, we needed even to apply a double Gaussian fit. The fitted functions were used to calculate number average molecular weight $M_n$ and polydispersity ($PDI$).

Figure S3: angular dependence of decay rate $\Gamma$ obtained by CONTIN analysis of DLS data for 0.12 g/l PEO114-(PDMAEMA40)3-PEO4 and PEO114-(PDMAEMA40)3-PEO114 in 0.1 M NaCl, pH 8 buffer and 2.5 · 10^{-3} M of [Co(CN)6]^{3-} at 10 °C; inset shows plot with the same scale for both dependences.
Figure S4: Exemplary intensity weighted size distributions at 80 °C (173°, Malvern Particle Sizer) for micelles of PEO114-(PDMAEMA40)4-PEO114 (red) and PEO114-(PDMAEMA55)3-PEO114 (black); 0.12 g/L polymer in 0.1 M NaCl with 2.5 mM [Co(CN)₆]³⁻ in pH 8 buffer.

Additional Comments to Self Assembly

The angular dependence of the Dynamic Light Scattering Data was investigated at 10 °C (Figure S3). The linear dependence of the decay rate against squared scattering vector emanating basically from the origin indicates low polydispersity of isotropic particles, which is consistent with the presence of spherical, star-shaped micelles as well as spherical vesicles.

Further, the size distributions of the micelles at 80°C were extracted by use of a particle sizer (Malvern Instruments), since our classical light scattering setup (Brookhaven Instruments) does not tolerate heating to such high temperatures. Though the backscattering optics prevents extraction of more detailed data, trends can be easily seen. Therefore we could detect monomodal size distribution for PEO114-(PDMAEMA55)3-PEO114 with hydrodynamic radius <R_h> close to 50 nm. This indicates that the aggregate size for is polymer is considerably smaller compared to the large vesicles obtained at low temperature. This is in accordance to collapsed, dense PDMAEMA domain instead of having a water-swollen PDMAEMA phase at low temperature. The gel-like PDMAEMA has much higher spacial demands, leading to the formation of large vesicles. At 80 °C, much smaller aggregates are obtained (most likely small vesicles; the hydrodynamic radius of 50 nm would however still allow the presence of spherical, star-shaped micelles). In contrast PEO114-(PDMAEMA₄₀)₄-PEO114 gives a bimodal distribution, resembling the situation present in the cryo-TEM images (Figure 4, main part). The smaller fraction, which is the major fraction in mass and number, gives only a <R_h> close to 5 nm. This is again considerably smaller compared to the size of the micelles at low temperatures. However the size is close to the core value seen in cryo-TEM, indicating good draining of the corona of the compact micelles.

References