Supplementary Material (ESI) for Chemical Communications

Supplementary Information for "Shell-crosslinked micelles from amphiphilic AB and ABA block copolymers of styrene-*alt*-(maleic anhydride) and styrene: polymerization, assembly and stabilization in one pot"

Materials. Styrene (99 %) was purchased from Sigma Aldrich (St Louis, MO); inhibitor was removed by passing over a column of basic alumina prior to use. Maleic anhydride (99 %), dodecanethiol (>98 %), tetrabutylammonium bromide (98 %), carbon disulfide (99.9 %), acetone (99.5 %), chloroform (99.8 %), 2-bromopropionic acid (99 %) and 1,2-ethylenedioxy bis(2-ethylamine) (98 %) were obtained from Sigma Aldrich and used without further purification. AIBN was recrystallized from methanol before use. All other solvents and reagents were obtained from Sigma Aldrich and used without further purification.

Measurements. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury 300 MHz spectrometer. IR spectra were obtained on a Perkin Elmer BX FT-IR system equipped with a diffuse reflectance accessory. Elemental analysis was performed by M-H-W Laboratories (Phoenix, AZ). Glass transition temperatures (T_g) were measured by differential scanning calorimetry (DSC) at a heating rate of 10 °C/min on a Mettler Toledo DSC822^e using Mettler Toledo Star SW 7.01 software. T_g values were taken at the midpoint of the inflection tangent on the third heating scan.

Gel Permeation Chromatography. Polymer molecular weight distributions were determined by gel permeation chromatography, conducted on a Waters Chromatography

Inc. model 150-CV, equipped with a model 410 DRI detector, a Precision Detectors PD2040 dual angle (15° and 90°) light scattering detector and a three-column series of Polymer Laboratories PLgel 10 μm mixed B 300 × 7.5 mm columns. The system was equilibrated at 35 °C in anhydrous THF, which served as the polymer solvent and eluent (flow rate 1.00 mL/min). Data collection and analysis was performed using Precision Detectors software. Interdetector delay volume and the light scattering detector calibration constant were determined using a nearly monodisperse polystyrene calibrant (Pressure Chemical Co., M_p = 90 000 g.mol^{-1}, $M_w/M_n < 1.04$). The differential refractometer was calibrated with standard polystyrene reference material (SRM 706 NIST) of known specific refractive index increment (dn/dc = 0.184 mL/g). The dn/dc values of the analyzed polymers were then determined from the differential refractometer response.

Dynamic Light Scattering. Particle size distributions were determined on a Brookhaven Instruments Co. dynamic light scattering system, consisting of a model BI-9000T digital goniometer, a model EMI-9865 photomultiplier, and a model 95-2 Ar laser (Lexel Corp.) operated at 514.5 nm. Measurements were made at 20 ± 1 °C. Prior to analysis, solutions were centrifuged in a model 5414 microfuge (Brinkman Inst. Co.) for 4 min to remove dust particles. Scattered light was collected at a fixed angle of 90 °C. The digital correlator was operated with 522 channels, an initial delay of 1.4 μs, a final delay of 10 ms, and a duration time of 15 minutes. A photomultiplier aperture of 400 μm was used. Only measurements for which the measured and calculated baselines of the intensity autocorrelation function agreed to within ± 0.1% were used to calculate nanoparticle diameter distributions. All determinations were made in triplicate. The calculations of the nanoparticle diameter distributions were performed by cumulants analysis using the ISDA software package (Brookhaven Instruments Co.).

Transmission Electron Microscopy. Carbon-coated copper grids were prepared by oxygen plasma treatment to make the surface hydrophilic. Particle samples were diluted 9:1 in water. A drop of diluted sample was deposited onto a grid; after 1 minute excess solvent was wicked away. A drop of 1% phosphotungstic acid (PTA) stain was then added, and left for 1 minute before excess stain was wicked away. Particle diameters and standard deviations were calculated from measurements of a minimum of 100 particles from three TEM micrographs from different regions.

Atomic Force Microscopy. Tapping-mode atomic force microscopy measurements were conducted in air with a Nanoscope III BioScope system (Digital Instruments) operated under ambient conditions with standard silicon tips (type: OTESPA-70; *L*: 160 µm; normal spring constant: 50 N/m; resonance frequency 246-282 kHz). Samples were diluted 99:1 in water, then a 10 µL drop of the solution was deposited onto a freshly-cleaved mica surface and allowed to evaporate. The average particle heights were calculated from the section analysis of at least 50 particles selected from at least 3 different regions. Typical AFM micrographs for micelles and crosslinked particles are shown in Figures S1 and S2 respectively.

Synthesis of S-1-dodecyl S'-(α,α-dimethylacetic acid) trithiocarbonate, 1. The compound was synthesized according to the method of Lai *et al.*[S1] KOH (2.5 g dissolved in 2.5 mL H_2O) was added to a solution of dodecanethiol (10.1 g, 0.050 mol) and tetrabutylammonium bromide (0.64 g, 2 mmol) in 25 mL of acetone. Carbon disulfide (2.6 mL) was added dropwise and the resulting orange-red solution was was stirred 30 min before addition of chloroform (5 mL). KOH (12.0 g dissolved in 10 mL H_2O) was added dropwise with vigorous stirring at 0 °C and the mixture was stirred overnight at room temperature. DI H_2O (60 mL) was then added and the mixture was acidified with 10 mL conc. HCl. Excess acetone was removed *in vacuo* and the solid product was separated by filtration and dissolved in hot isopropanol. On cooling, bis(dodecyl) trithiocarbonate crystallized out and was filtered off. The isopropanol was removed *in vacuo* and the resulting oil was recrystallized from hexanes, yielding 9.20 g yellow crystals, m. p. 63 °C (lit. 62-3 °C).[S1] Yield: 60 %.

^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 0.89 (*t*, 3H, J = 6.75 Hz, $C\underline{H}_3(CH_2)_{11}$-), 1.2-1.5 (*m*, 18H, $CH_3(C\underline{H}_2)_9CH_2CH_2S$-), 1.68 (*quint.*, 2H, J = 7.5 Hz, -$C\underline{H}_2CH_2S$-), 1.73 (*s*, 6H, $(CH_3)_2C$<), 3.29 (*t*,2H, J = 7.5 Hz, -CH_2S-) ppm. ^{13}C NMR ($CDCl_3$): 14.4 ($\underline{C}H_3(CH_2)_{11}$-), 22.9 ($CH_3\underline{C}H_2$-), 25.4 (($\underline{C}H_3)_2C$<), 28.0, 29.2, 29.3, 29.6, 29.7, 29.8, 29.9, 32.1 (dodecyl carbons), 37.3 (-$\underline{C}H_2S$-), 55.8 ($HOOC(CH_3)_2\underline{C}S$-), 178.8 (-COOH), 221.0 (-SC(=S)S-) ppm. IR (diffuse reflectance): 2921, 2850, 1714, 1281, 1070, 813 cm^{-1}. MS: 365.164 $[M + H]^+$. Elemental Analysis: C 54.25 H 8.21 S 25.14. Theoretical: C 56.00 H 8.85 S 26.38.

Synthesis of bis(2-propionic acid) trithiocarbonate, 2.

2-Bromopropionic acid (4.7 mL, 50 mmol) was added slowly to a suspension of CS_2 (6.0 mL, 110 mmol) in a solution of 6.6 g KOH in 150 mL H_2O. The yellow solution was stirred 48 h at room temperature, then extracted twice with 20 mL $MeCl_2$ to remove excess CS_2. The yellow aqueous layer was acidified with conc. HCl and extracted with $MeCl_2$ (5 x 20 mL). The combined organic layers were dried over $MgSO_4$ and solvent was removed *in vacuo* to give 4.6 g yellow solid. This was recrystallized from acetone/hexanes to give 3.44 g yellow crystals, m. p. 90 °C. Yield: 51.8 %

^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 0.89 (*t*, 3H, J = 6.75 Hz, $C\underline{H}_3(CH_2)_{11}$-), 1.2-1.5 (*m*, 18H, $CH_3(C\underline{H}_2)_9CH_2CH_2S$-), 1.68 (*quint.*, 2H, J = 7.5 Hz, -$C\underline{H}_2CH_2S$-), 1.73 (*s*, 6H, $(CH_3)_2C$<), 3.29 (*t*,2H, J = 7.5 Hz, -CH_2S-) ppm. ^{13}C NMR ($CDCl_3$): 14.4 ($\underline{C}H_3(CH_2)_{11}$-), 22.9 ($CH_3\underline{C}H_2$-), 25.4 (($(\underline{C}H_3)_2C$<), 28.0, 29.2, 29.3, 29.6, 29.7, 29.8, 29.9, 32.1 (dodecyl carbons), 37.3 (-$\underline{C}H_2S$-), 55.8 ($HOOC(CH_3)_2\underline{C}S$-), 178.8 (-COOH), 221.0 (-SC(=S)S-) ppm. IR (diffuse reflectance): 2921, 2850, 1714, 1281, 1070, 813 cm^{-1}.

MS: 254.982 $[M + H]^+$. Elemental Analysis: C 33.14 H 4.20 S 37.59. Theoretical: C 33.06 H 3.96 S 37.82.

Polymerizations. Styrene, maleic anhydride, **1** or **2** and AIBN (20 mol% relative to RAFT agent) were mixed in appropriate ratio with dioxane (20 mL) and degassed by purging with N_2 for 10 min. In a typical experiment, 15 g STY, 5 g maleic anhydride, 183 mg **1** and 16 mg AIBN were used. The mixtures were heated in an oil bath set to 60 °C for 21 h. A small sample was taken in order to estimate conversion by NMR spectroscopy, 20 mL of THF was added and the resulting solutions were precipitated into 1L Et_2O in order to isolate the polymer, which was subsequently dried under vacuum at 120 °C. Molecular weight distributions were measured by GPC, compositions were calculated by elemental analysis. Characterization data is given in Table 1 of the main text. Dynamic scanning calorimetry of the diblock polymers **3-5** revealed two glass transitions for each, at approximately 78 and 170 °C (Table S1). The triblock copolymer **6** also showed two glass transitions, at 115 and 164 °C respectively. Polymers exhibited characteristic IR absorbances at 1856 and 1776 cm^{-1} (coupled anhydride C=O stretches), 1494 and 1453 cm^{-1} (aromatic C=C ring stretches) and 699 cm^{-1} (aromatic out of plane C-H bending). A typical IR spectrum (for diblock copolymer **5**) is shown in Figure S3A.

Table S1. Glass transition temperatures for polymers **3-6**.

Polymer	**T$_{g1}$ (°C)**	**T$_{g2}$ (°C)**
3	77.6	172
4	76.5	180
5	76.8	170
6	115	164

Pyrolysis of 6. A 10.0 mg sample of **6** was heated at 250 °C for 30 minutes under a stream of nitrogen in a Perkin-Elmer TGA/SDTA851^{e} thermogravimetric analysis system. This treatment resulted in a mass loss of 0.7 mg (7 %). The residue was dissolved in THF and its molecular weight distribution was analyzed by GPC (Figure S4).

Formation of Micelles. Block copolymer (0.1 g) was dissolved in 50 mL THF, to which was added one drop (~10 μL) triethylamine. Nanopure (> 18 MΩ.cm^{-1}) water (50 mL) was added at 10 mL/hour with stirring. A further 50 mL water was then added in one portion, and the mixture was dialyzed (6000-8000 MWCO tubing) vs. DI water for several days to remove THF. More water was then added to reach a final volume of 200 mL (0.5 mg/mL). The final suspensions ranged in appearance from colorless to bluish-white.

Crosslinking. To a micellar solution of block copolymer (0.5 mg/mL) was added 1,2-ethylenedioxy bis(ethylamine) (10 mol% relative to maleic anhydride units). After stirring 20 minutes, EDC was added (20 mol% relative to maleic anhydride units). The solution was stirred overnight, then dialyzed for 3 days against DI water to remove unreacted crosslinker and urea byproducts (6000-8000 MWCO tubing).

Confirmation of crosslinking. Formation of amide bonds was confirmed by IR spectroscopy of lyophilized sample (Figure S3C), with the appearance of a new absorption at 1636 cm^{-1}, corresponding to the amide C=O stretch. To demonstrate the formation of a crosslinked network, both the crosslinked particle suspensions and their non-crosslinked precursors were diluted with 9 parts THF. The resulting suspensions were analyzed by DLS (Figure S5). The absence of scattering for non-crosslinked samples of **3-5** indicated that these polymers formed a molecular solution in this solvent mixture. Their crosslinked analogs, however, did not dissociate under this treatment. Dilution with THF was not sufficient to completely dissociate the triblock copolymer **6**, but a clear difference in scattering intensity between non-crosslinked and crosslinked samples of **6** was observed (Figure S5D).

One-pot copolymer synthesis, micellization and crosslinking to produce shell-crosslinked nanoparticles. MA (0.5 g, 5.1 mmol) and STY (1.5 g, 14.4 mmol) were heated at 60 °C in the presence of 2.0 g dioxane, **1** (18 mg, 49 µmol), and a trace of AIBN, for a period of 16 hours under N_2. The resulting mixture (conversion = 89 % by NMR, theoretical M_n = 36300 $g.mol^{-1}$) was dissolved in 1 L of THF. A 250 mL aliquot of the solution was taken, to which 1,2-ethylenedioxy bis(2-ethylamine) (18.9 mg, 128 µmol, 10% relative to MA) was added with vigorous stirring, followed immediately by 250 mL nanopure water. The THF was removed by evaporation *in vacuo* (at RT) and the micellar solution was diluted to 1 L with nanopure water. The resulting mixture contained nanoparticles with D_h 164 ± 2 nm, and a polydispersity of 0.08 ± 0.03 (measured by DLS). AFM analysis of the nanoparticles gave an average height of 118 nm and standard deviation of 38 nm. The persistence of these particles in 9:1 THF:H_2O indicated that crosslinking had taken place (Figure S7). As a control, the procedure was repeated with a second 250 mL aliquot of the polymer solution in THF, using triethylamine (25.9 mg, 256 μmol, 10% relative to MA) in place of 1,2-ethylenedioxy bis(ethylamine). DLS of the resulting solution revealed the presence of nanoparticles with average diameter 137 ± 5 nm, and polydispersity of 0.20 ± 0.02 (measured by DLS). On dilution with 9 parts THF, these particles dissociated to form a molecular solution (Figure S7)

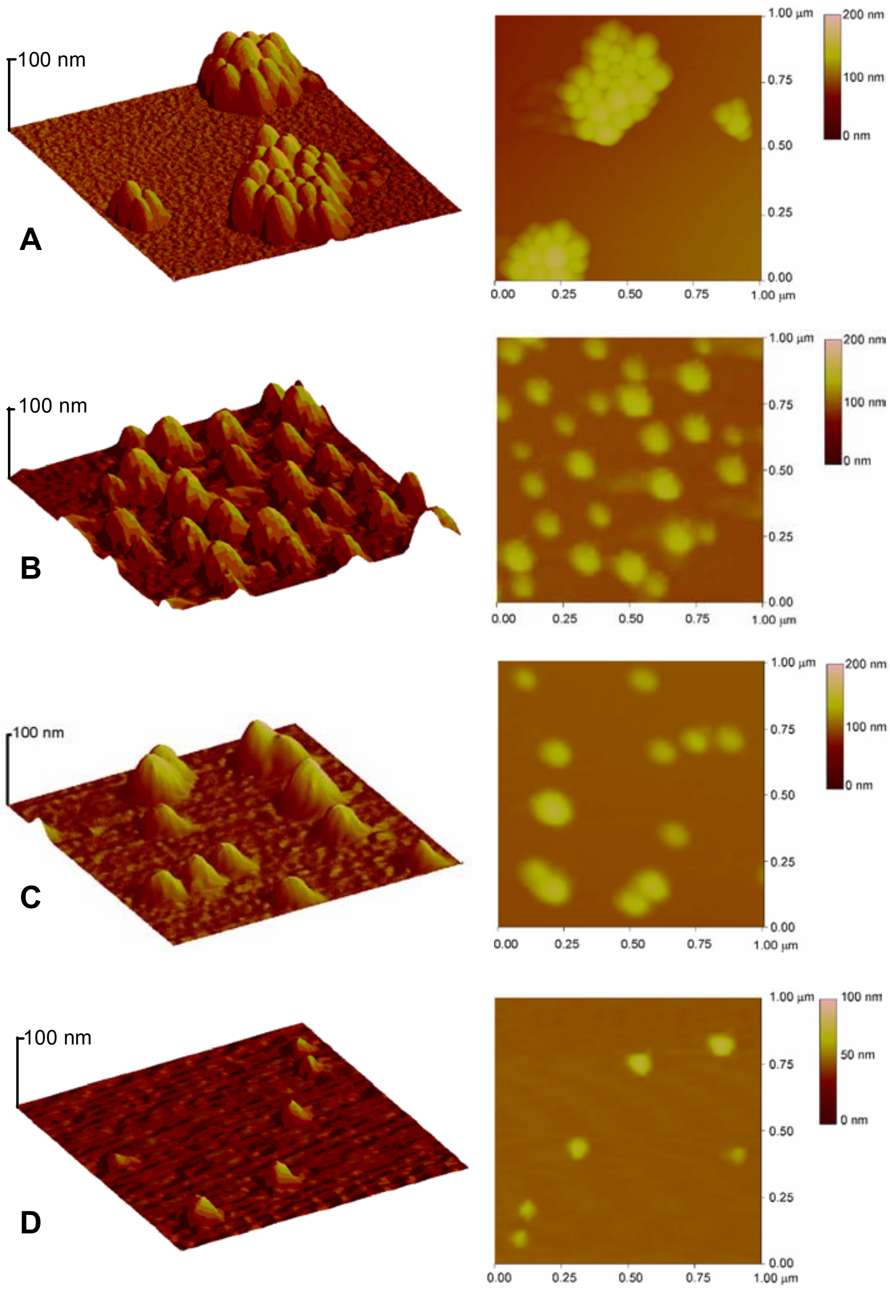

Figure S1. Atomic force micrographs of 1 µm × 1 µm areas of micelles of **3** (A), **4** (B), **5** (C), and **6** (D) deposited on a freshly cleaved mica surface.

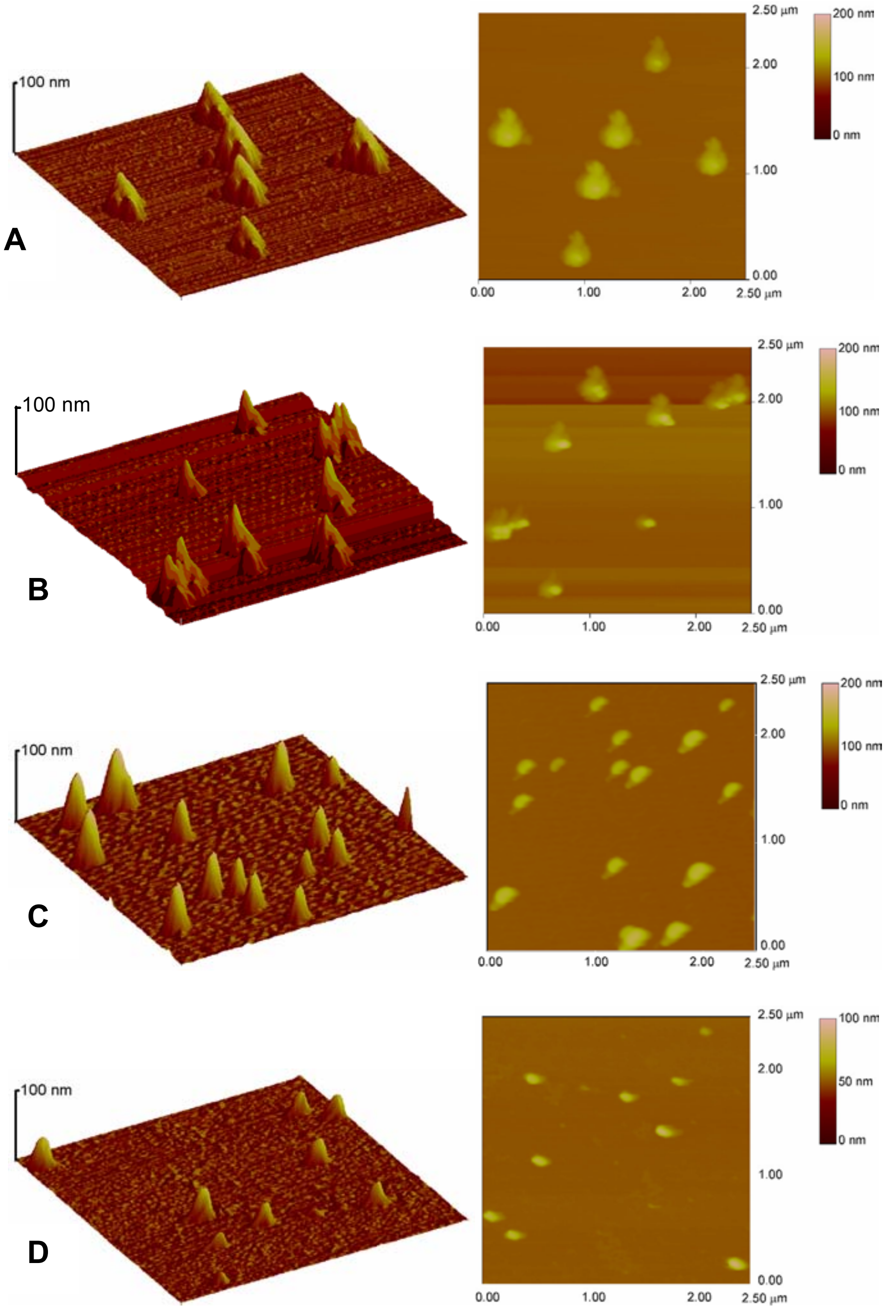

Figure S2. Atomic force micrographs of 2.5 µm × 2.5 µm areas of crosslinked micelles of **3** (A), **4** (B), **5** (C), and **6** (D) deposited on a freshly cleaved mica surface.

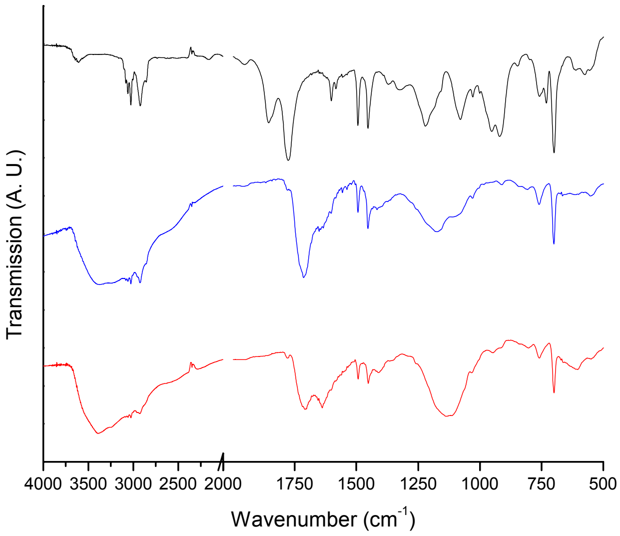

Figure S3. IR spectra of diblock copolymer **5**: (A) before hydrolysis; (B) after micellization and lyophilization; (C) after micellization, crosslinking and lyophilization.

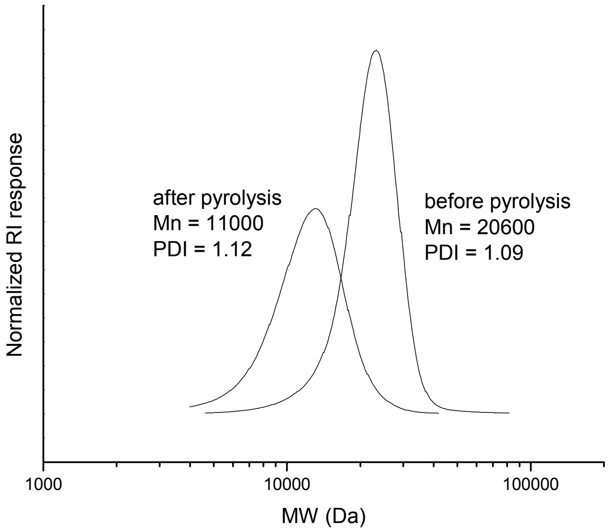

Figure S4. Molecular weight distributions of triblock copolymer 6, before and after pyrolysis at 250 °C for 30 minutes. Distributions are normalized to equal area.

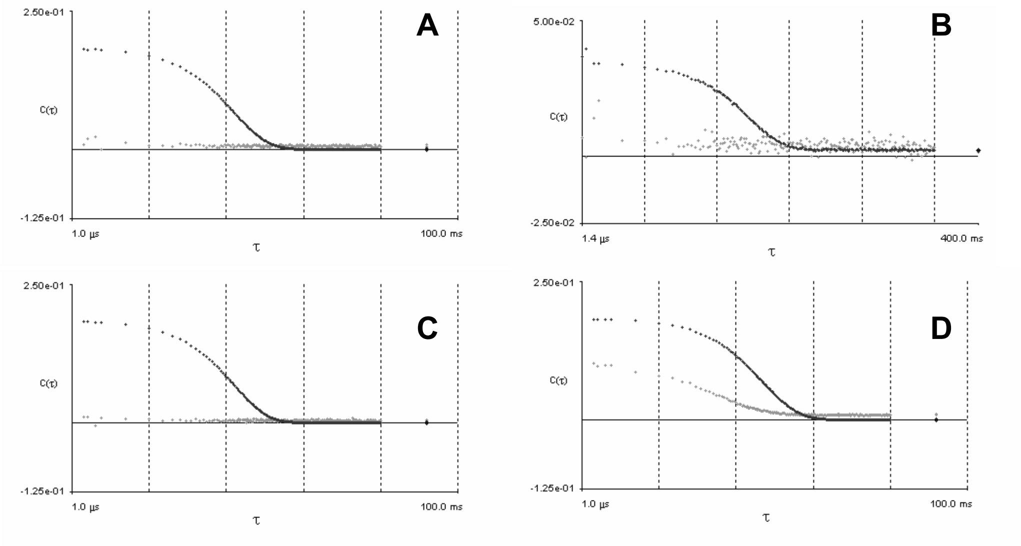

Figure S5. DLS correlation functions for crosslinked (black) and non-crosslinked (gray) micelle solutions of **3-6** (A-D respectively) after dilution with 9 parts THF (Final concentration: 0.05 mg/mL).

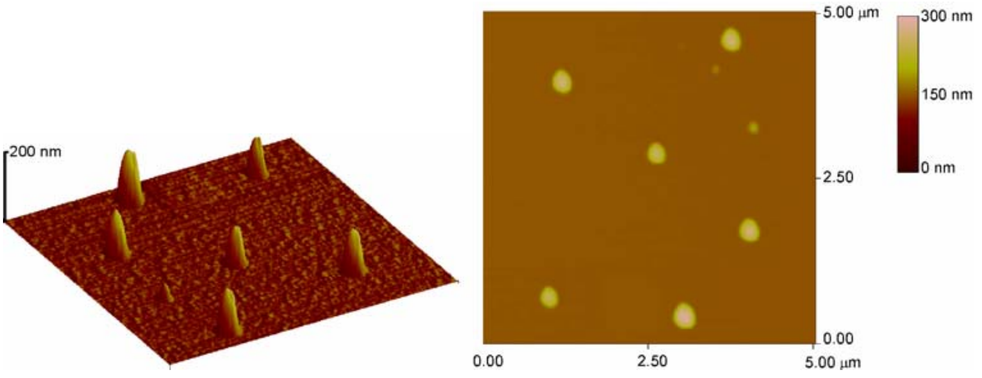

Figure S6. Atomic force micrograph of a 5 µm × 5 µm area of one-pot crosslinked nanoparticles, deposited onto a freshly-cleaved mica surface.

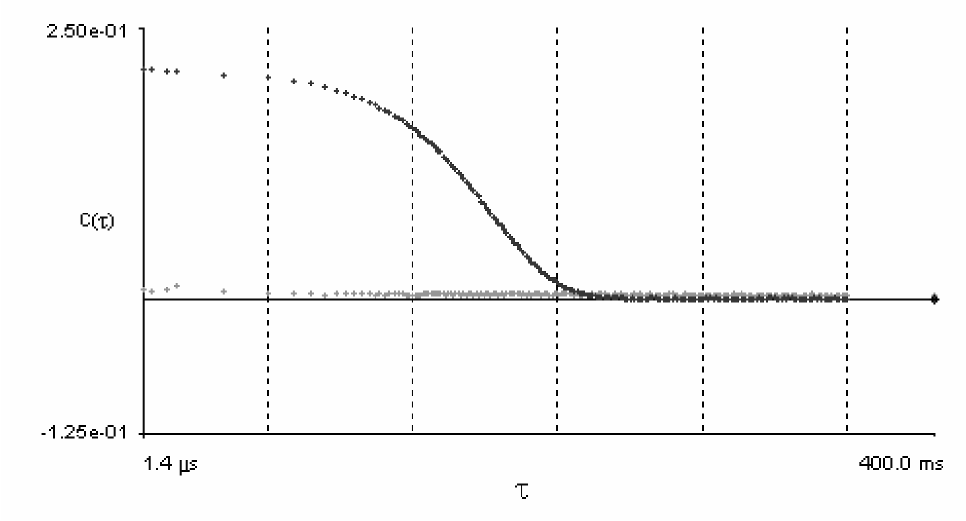

Figure S7 DLS correlation functions for crosslinked (black) and non-crosslinked (gray) one-pot micelles and crosslinked nanoparticles after dilution with 9 parts THF (Final concentration: 0.05 mg/mL).

References.

S1. J. T. Lai, D. Filla and R. Shea, *Macromolecules*, 2002, **35**, 6754.