Aggregation-free and high stability core-shell polymer nanoparticles with high fullerene loading capacity, variable fullerene type, and compatibility towards biological conditions

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Synthesis. Small molecule synthesis and characterization details: Reactions requiring anhydrous and inert conditions were carried out under an argon atmosphere using oven-dried glassware with standard Schlenk-technique. Solvents were degassed by sonication and bubbling nitrogen through. All other chemicals and solvents were obtained commercially and used as received. Column chromatography was carried out on silica gel 40−63 mesh. Reactions were monitored by thin-layer chromatography (TLC) on silica gel-coated aluminium plates (60 F254, Merck) and visualized with UV light (λ = 254 and 365 nm). Purity of the reported compounds was established by comparing the integrals of the peaks of the desired compounds to the ones arising from impurities. ^{1}H NMR spectra were recorded at 396 or 400 MHz JEOL ECA instruments (and the corresponding frequencies for ^{13}C) in $CDCl_3$ unless otherwise noted. Chemical shifts are given in ppm and coupling constants in Hz ($CDCl_3$ ^{1}H: 7.26 ppm, ^{13}C: 77.23 ppm; $C_2D_2Cl_4$ ^{1}H: 5.99 ppm, ^{13}C: 73.78 ppm). For fullerenene studied with NMR, ^{13}C-enriched C_{60} and C_{70} was purchased from io-li-tec and used as received. High-resolution mass spectra (HRMS) were recorded by using Waters Q-Tof Premier spectrometer in ESI+ mode with TOF mass analyser.

Methyl corannulene sulfide: To a degassed solution of sodium methanethiolate (128 mg, 1.82 mmol) in anhydrous DMI (4 mL) was added monobromocorannulene (400 mg, 1.21 mmol) and the mixture was heated to 60 °C overnight under argon. After cooling to room temperature, it was diluted with 150 mL toluene, washed with 80 mL 2M HCl, 2x80 mL water and 80 mL brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified using column chromatography on silica gel eluting with hexane:DCM 95:5. A yellow solid was obtained: 262 mg (0.88 mmol) 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.8 Hz, 1H), 7.95 – 7.73 (m, 6H), 7.68 (d, J = 8.8 Hz, 1H), 7.58 (s, 1H), 2.71 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.23, 136.18, 135.87, 135.77, 134.54, 131.34, 131.08, 131.00, 130.67, 130.56, 127.64, 127.58, 127.27, 127.10, 126.99, 126.45, 125.45, 122.89, 17.28. HRMS calcd for $C_{21}H_{13}S$ (MH^+) 297.0738 found 297.0753 ESI+).

Mercaptocorannulene: To degassed solution of corannulene-methyl sulfide (600 mg, 2 mmol) in anhydrous DMF (12 mL) was added sodium methanethiolate (280 mg, 4 mmol) under argon. The mixture was heated to 145°C for 1 hour (until TLC indicated the consumption of the starting material). The cooled mixture was quenched with 5 mL degassed 1M aq. HCl and poured into 100 mL of water. It was extracted with 2x50 mL toluene, and the combined organic layers were washed with 3x50 mL water and 20 mL brine. The organic

layer was dried on anhydrous Na_2SO_4, filtered, and concentrated. Corannulene thiol was obtained as a yellow solid: 521 mg (1.86 mmol), 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.8 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.84 – 7.76 (m, 6H), 7.68 (d, *J* = 8.8 Hz, 1H), 3.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.96, 135.78, 135.61, 134.78, 131.23, 131.13, 131.01, 130.74, 129.27, 127.85, 127.73, 127.72, 127.44, 127.35, 127.20, 127.12, 126.32, 125.47. HRMS calcd. For $C_{20}H_{11}S$ (MH^+) 283.0581 found 283.0575 (ESI+).

Polymer and nanoparticle synthesis and characterization details: glycidyl methacrylate, 4,4'-dinonyl-2,2'-dipyridyl (dNbpy), Cu(I)Br, thiophenol, sodium borohydride ($NaBH_4$), fullerene-C_{60}, fullerene-C_{70}, adipic acid, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 4-dimethylaminopyridine (DMAP), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sheep's red blood, bovine serum albumin (BSA), Bradford reagent were purchased from commercial sources. PEG macroinitiator (5 kDa) was prepared according to the procedure described in *Polymer chemistry*, 2012, *3*, 2342. NMR spectra were recorded on a Varian NMR system 500 MHz spectrometer, using DMSO-d_6 as the solvents. Gel permeation chromatography (GPC) (against polystyrene standards) was carried out in THF using a Waters system (Waters 1515 pump, Water 2414 refractive 5 index detector) instrument with three styragel HR 0.5, HR 2, HR 4 columns. Transmission electron microscope (TEM) operating at an accelerating voltage of 200 kV was employed for imaging on a Tecnai 20 from FEI. For TEM, the samples were prepared by dropping a solution on the TEM grids and drying under ambient conditions. The particle size was characterized by dynamic light scattering (DLS) at a fixed angle of θ = 165° using a ELSZ-2000 from Otsuka Electronics. The UV/Vis measurements were carried out on a Lambda 265 UV/Vis-spectrometer from Perkin-Elmer. The fluorescence emission was carried out on a F-7000 FL Spectrophotometer from Hitachi.

PEG-*b*-PGMA: PEG-Br macroinitiator (5 kDa) (250 mg, 0.05 mmol), glycidyl methacrylate (500 mg, 3.52 mmol), 4,4'-dinonyl-2,2'-dipyridyl (dNbpy) (40.9 mg, 0.05 mmol), and anisole (1 mL) added to a schlenk tube and was purged by bubbling Ar for 30 min. Cu(I)Br (7.2 mg, 0.1 mmol) was added and Ar purging was continued for another 5 min. The reaction mixture was then stirred under inert atmosphere at room temperature for 3 hrs. After this time, the reaction mixture was cooled to room temperature and precipitated into cold isopropanol, filtered, and passed through a small plug of silica gel using THF as an eluent. The organic solvent was reduced under low pressure and then precipitated into isopropanol. The obtained white powder was then dried under high vacuum conditions. ^{1}H NMR (500 MHz, $CDCl_3$) δ

4.31 (br s, COO**CH$_2$**), 3.78 (br s, COO**CH$_2$**), 3.64 (br s, **CH$_2$CH$_2$**O), 3.37 (s, O**CH$_3$**), 3.23 (br s, CH$_2$**CH**CH$_2$O), 2.84 (br s, COOCH$_2$CH**CH$_2$**O), 2.64 (br s, COOCH$_2$CH**CH$_2$**O), 2.15 – 0.54 (br m, backbone). GPC(THF): M_n = 10600, M_w = 12700, PDI (M_w/M_n) = 1.20.

Block copolymer 1: To a solution of PEG-*b*-PGMA (30 mg, 0.06 mmol of epoxy units) and mercaptocorannulene (18 mg, 0.06 mmol (1.05 eq. per epoxy unit)) were dissolved in THF (0.9 mL) at 0 °C. Then, a solution of sodium borohydride (3 mg, 0.08 mmol (1.25 eq. per corannulene)) in water (0.1 mL) was added dropwise (caution: the reaction is exothermic and hydrogen gas evolves). The reaction mixture was stirred at room temperature for 2 hr. After this time, the reaction mixture was diluted with methanol and then dialyzed (dialysis tube: cutoff 1 kDa) against methanol for 1 day. The organic solvent was reduced under low pressure and polymer was precipitated into diethyl ether. The obtained yellow powder was then dried under high vacuum conditions. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 – 6.48 (br s, **Corannulene**), 4.10 (br s, COO**CH$_2$CH**(OH)), 3.65 (br s, **CH$_2$CH$_2$**O), 3.38 (s, O**CH$_3$**), 3.20 (br s, CH(OH)**CH$_2$**S), 2.16 – 0.47 (br m, backbone). GPC(THF): M_n = 14500, M_w = 17800, PDI (M_w/M_n) = 1.23.

Block copolymer 2: To a solution of PEG-*b*-PGMA (30 mg, 0.06 mmol of epoxy units) and thiophenol (7 mg, 0.06 mmol (1.05 eq. per epoxy unit)) were dissolved in THF (0.9 mL) at 0 °C. Then, a solution of sodium borohydride (3 mg, 0.08 mmol (1.25 eq. per corannulene)) in water (0.1 mL) was added dropwise (caution: the reaction is exothermic and hydrogen gas evolves). The reaction mixture was stirred at room temperature for 2 hr. After this time, the reaction mixture was precipitated into cold methanol and hexane thrice. The obtained white powder was then dried under high vacuum conditions. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.06 (br s, **Ar**), 4.09 (br s, COOCH$_2$**CH**(OH)), 3.94 (br s, COO**CH$_2$**CH(OH)),), 3.63 (br s, **CH$_2$CH$_2$**O), 3.37 (s, O**CH$_3$**), 3.00 (br s, CH(OH)**CH$_2$**S), 2.13 – 0.74 (br m, backbone). GPC(THF): M_n = 13200, M_w = 16100, PDI (M_w/M_n) = 1.22.

Critical micelle concentration determination. Micelle solutions ranging from 3 μg/mL to 100 μg/mL were prepared. The CMC of **1** was determined via maximum fluorescence intensity of corannulene versus log polymer concentration (Ex = 300 nm/slit = 5 nm, Em = 360-600 nm/slit = 20 nm).

Determination of fullerene loading. Fullerene loading was calculated via the Lambert-Beer law using UV/Vis spectroscopy; $A = \varepsilon cl$ (A = absorbance of the samples, ε = molar absorbance coefficient, l: optical path length.) (molar absorption coefficient of C_{60} ε (340 nm) = 49,000 M^{-1} cm^{-1}; molar absorption coefficient of C_{70} ε (384 nm) = 34,000 M^{-1} cm^{-1}).

ABTS radical cation scavenging assay. 7.4 mM ABTS (5 mL PBS buffer pH 7.4) and 2.6 mM potassium persulfate (5 mL PBS buffer pH 7.4) were stirred at room temperature for 24 hr in the dark. The solutions of nanoparticles ranging from 5 μg/mL to 1000 μg/mL were prepared. The initial absorbance of ABTS radical cation and sample mixtures were adjusted to 0.706 ± 0.02 at 734 nm. After incubating for 3 mins, the absorbance of the mixtures was recorded by UV/Vis-spectrometer at 734 nm, respectively. The concentration of 50% inhibition was calculated by the following equation: $I = \{1-(A_i-A_j)/A_{control}\}x100$ (A_i = the absorbance of nanoparticles with ABTS solution, A_j = the absorbance of pure nanoparticles, $A_{control}$ = the absorbance of pure ABTS solution without nanoparticles.)

Hemolysis assay. Hemolytic activity of the nanoparticles was determined using sheep's red blood cells (RBCs). RBCs were pelletized by centrifuging 1 mL of the blood and then washing the RBCs at 4 times with PBS buffer (pH 7.4) solution. The nanoparticles were re-dispersed in PBS buffer. Then, 1mL of nanoparticles and 25 μL of the RBCs were mixed and incubated at room temperature for 2h. After incubation, the mixtures were re-pelletized by centrifuging and 200 μL of the supernatant of each sample were put into wells in a 96-well plate. The absorbance at 540 nm was measured with a microplate reader. The percent hemolysis was calculated by following equation: $I = \{(A_i-A_{negative})/(A_{positive}-A_{negative})\}x100$ (A_i = the absorbance of nanoparticle solution, $A_{negative}$ = the absorbance of PBS solution, $A_{positive}$ = the absorbance of DI water.)

Protein adsorption. Protein adsorption of the nanoparticles was determined using bovine serum albumin (BSA). The nanoparticles were re-dispersed in PBS buffer (pH 7.4) (1 mg/mL). Then, 0.5 mL of nanoparticle solution and 0.5 mL of BSA (1 mg/mL) were mixed and incubated at room temperature for 3h to reach adsorption equilibrium. After incubation, the nanoparticles were removed by centrifugation at 14000 rpm for 40 mins and the supernatant was analyzed for BSA. The supernatant BSA concentrations were determined via the Bradford reagent using UV/Vis-spectrometer. 50 μL of the supernatants were added to Bradford reagent (950 μL). After incubation for 3 mins, the absorbance of the mixtures was recorded by UV/Vis-spectrometer at 595 nm. The concentrations of % BSA were calculated by the following equation: $I = \{1-(A_i-A_{control})\}x100$ (A_i = the absorbance of Bradford reagent with supernatants in the presence of nanoparticles, $A_{control}$ = the absorbance of Bradford reagent with supernatants without nanoparticles.)

DLS data for micelles of block copolymer **1** shown in Figure 3b.

	Solvent	Diameter	PDI
Micelle	water	37.8	0.209
Micelle	DMF	0.2	-
Micelle after crosslinking	water	31.7	0.212
Micelle after crosslinking	DMF	82.2	0.261

DLS data for C_{60}-loaded micelles of block copolymer **1** shown in Figure 6a.

	Solvent	Diameter	PDI
Micelle+C_{60}	water	106	0.261
Micelle+C_{60}	DMF	0.2	-
Micelle+C_{60} after crosslinking	water	92.3	0.274
Micelle+C_{60} after crosslinking	DMF	220	0.172

DLS data for C_{70}-loaded micelles of block copolymer **1** shown in Figure 6b.

	Solvent	Diameter	PDI
Micelle+C_{70}	water	135	0.291
Micelle+C_{70}	DMF	0.2	-
Micelle+C_{70} after crosslinking	water	99.9	0.301
Micelle+C_{70} after crosslinking	DMF	178	0.322

DLS data for micelles of block copolymer **2** shown in Figure S7.

	Solvent	Diameter	PDI
Micelle	water	25.0	0.168
Micelle+C_{60}	water	25.1	0.163
Micelle+C_{70}	water	30.5	0.178

DLS data for C_{60}-loaded micelles of block copolymer **1** shown in Figure S12-13.

	Solvent	Diameter	PDI
Micelle+C_{60} after crosslinking (6 month)	water	92.0	0.264
Micelle+C_{60} after crosslinking	pH 7.4 buffer	91.9	0.256
Micelle+C_{60} after crosslinking	pH 2.5 buffer	98.1	0.272

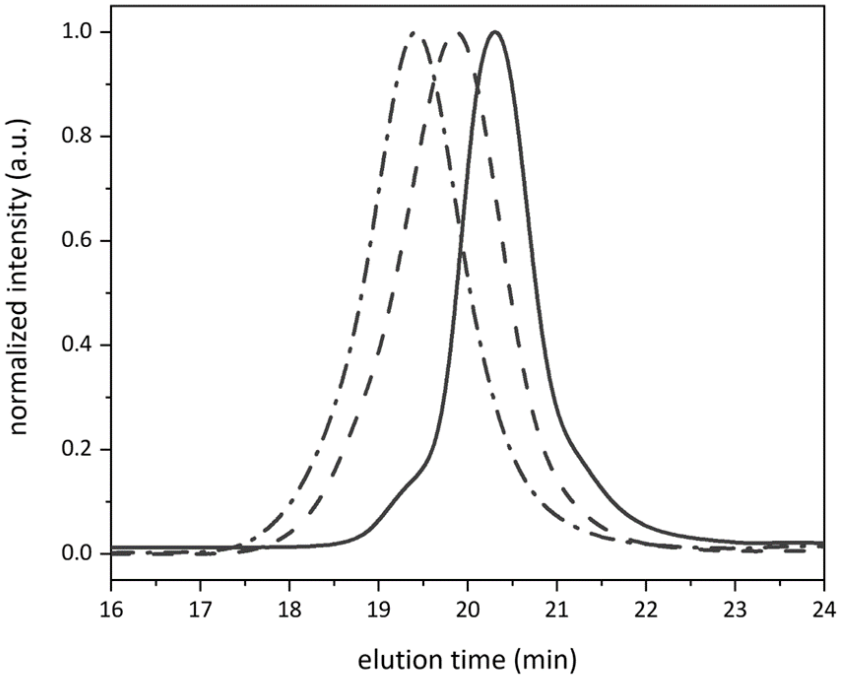

Figure S1. GPC (THF) traces of macroinitiator PEG-Br (solid line), PEG-PGMA block copolymer (dash line), and the host block copolymer **1** (dash-dot line).

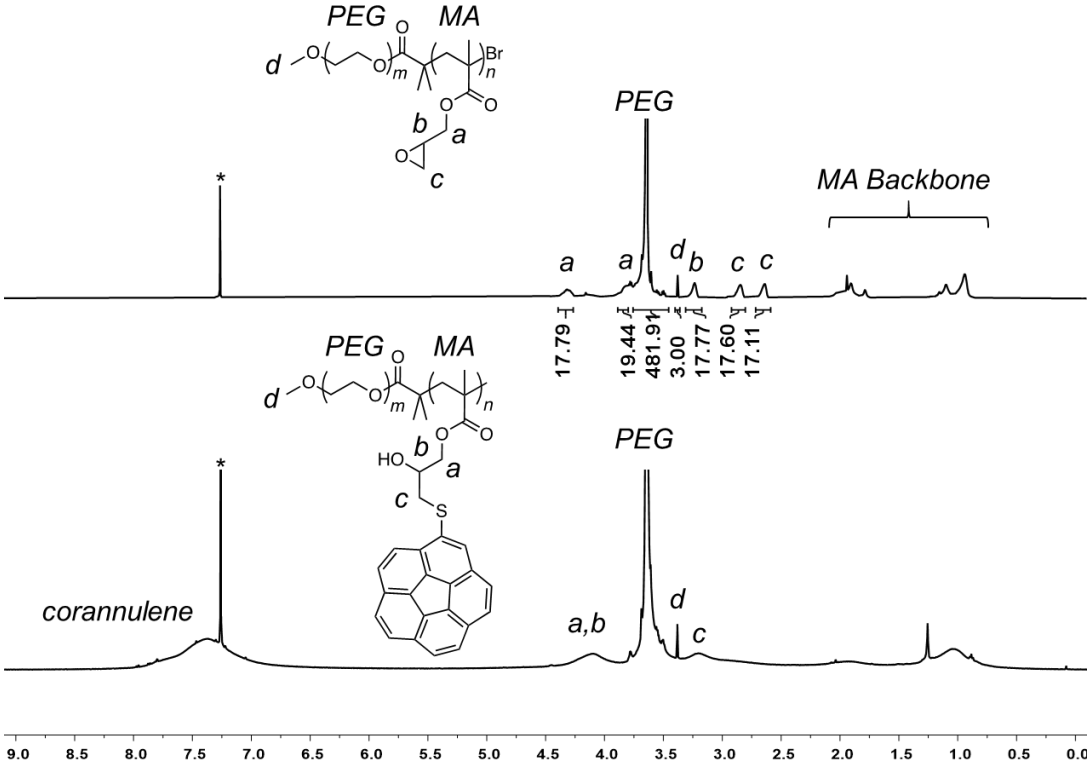

Figure S2. [1]H-NMR of PEG-PGMA block copolymer (top) and the host block copolymer **1** (bottom) in deuterated chloroform.

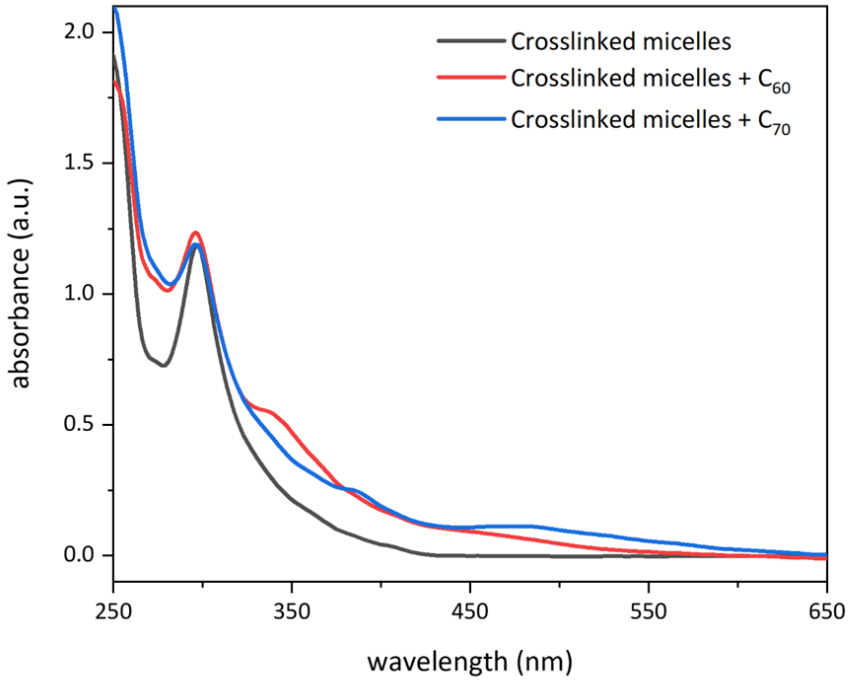

Figure S3. UV-Vis spectra of polymer **1** and core-crossliked polymer nanoparticles containing C_{60} and C_{70} in water.

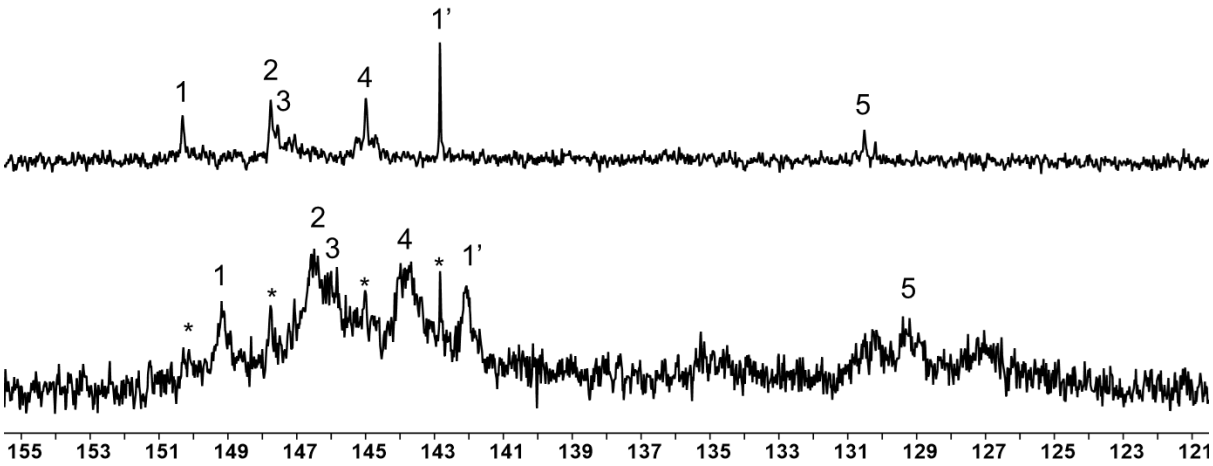

Figure S4. ^{13}C-NMR of $C_{70\,(1\text{-}5)}$/$C_{60\,(1')}$ (top) and polymer nanoparticles (bottom) in deuterated tetrachloroethane. The signals arising from the external standard are labeled with an asterisk.

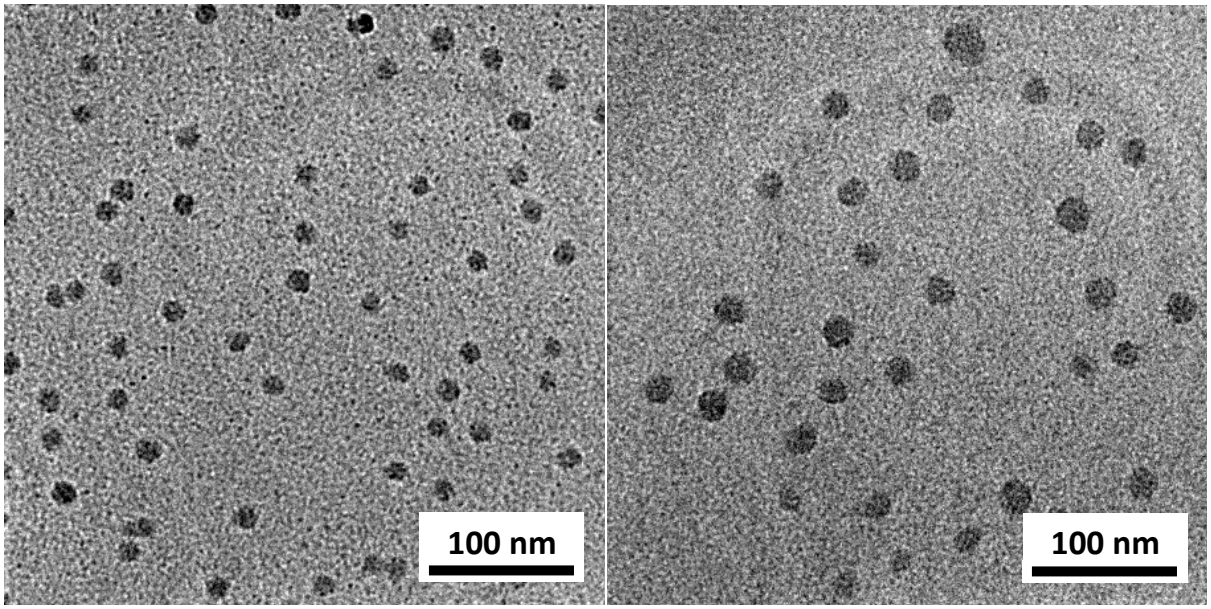

Figure S5. Transmission electron microscopy images of the fullerene C_{60} (left) and C_{70}-loaded (right) nanoparticle cores.

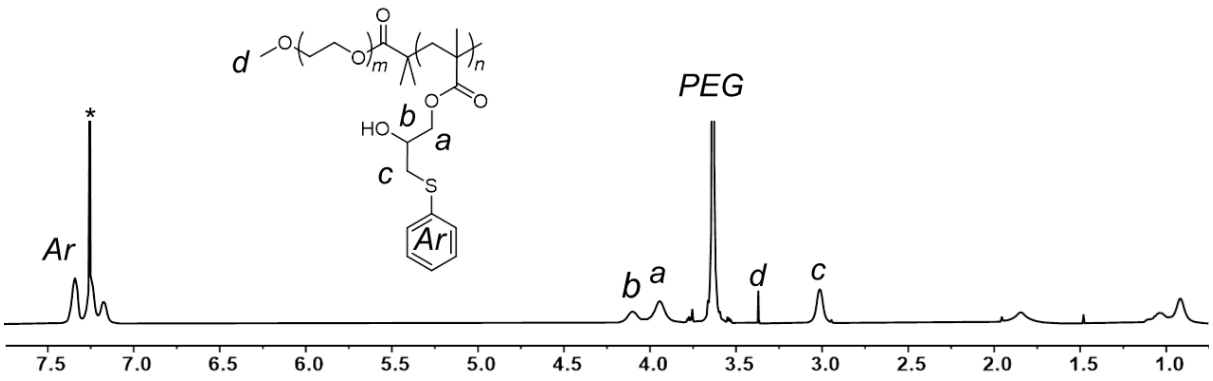

Figure S6. ^{1}H-NMR of the block copolymer **2** in deuterated chloroform.

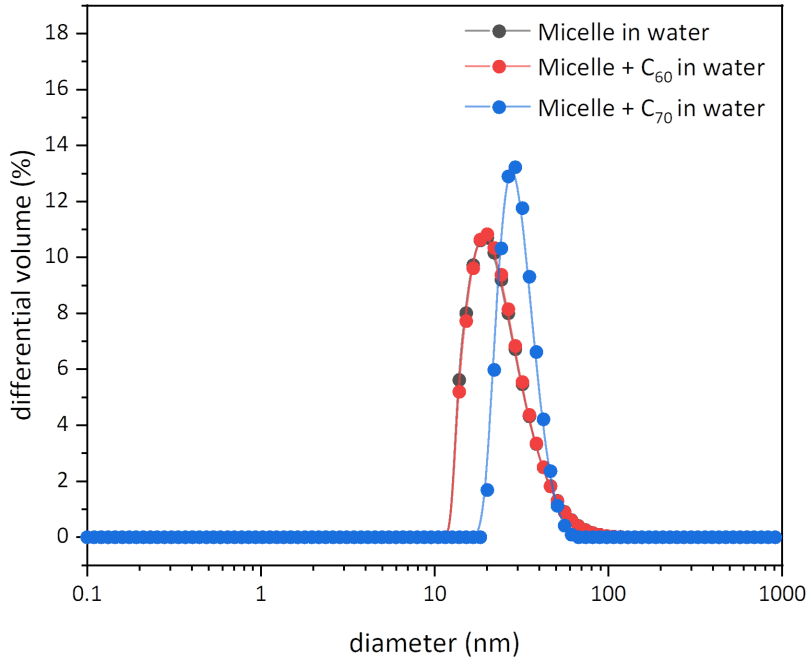

Figure S7. DLS data for block copolymer **2** in water in the presence or absence of fullerenes.

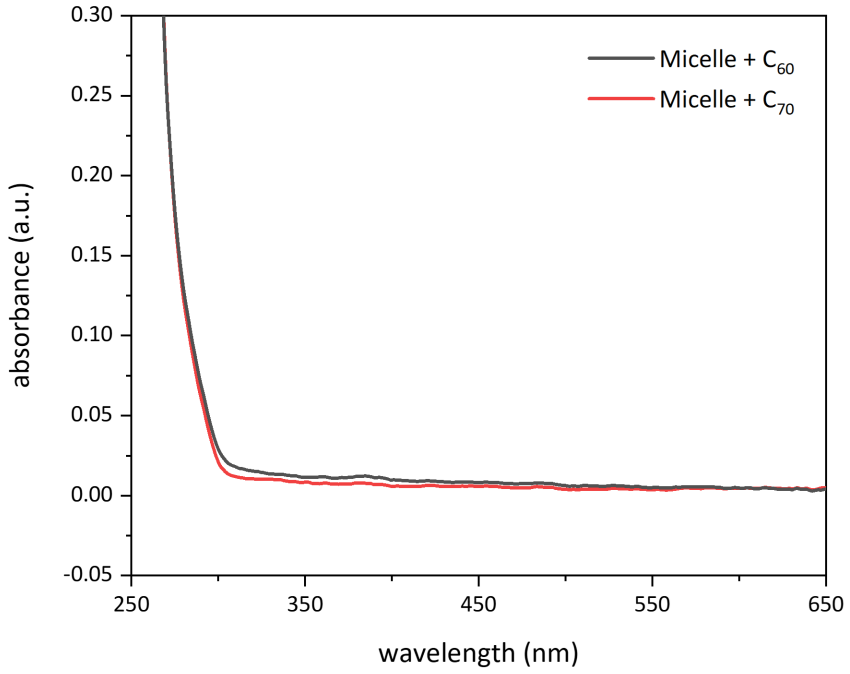

Figure S8. UV-Vis spectra of for block copolymer **2** and fullerene C_{60} and C_{70}.

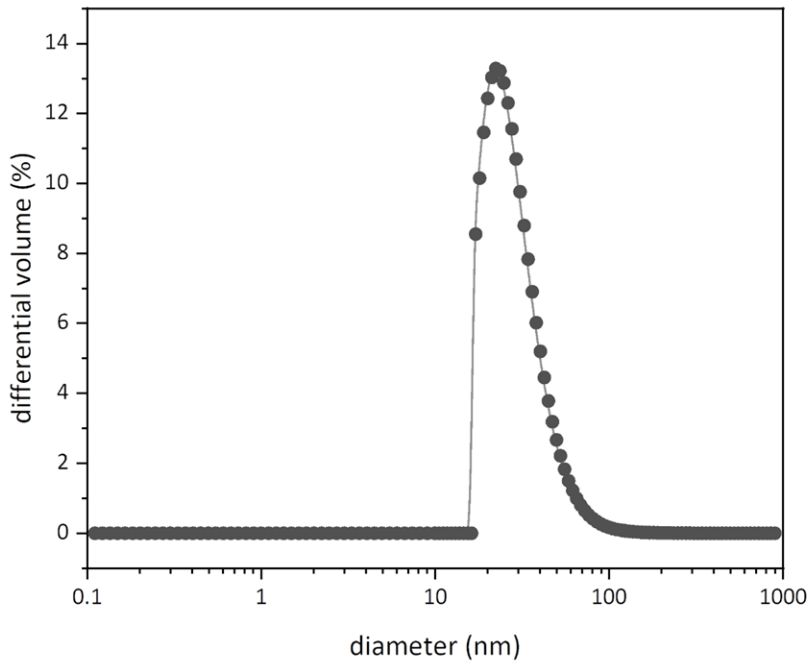

Figure S9. DLS data after freeze-drying and re-dispersing the core-crossliked polymer nanoparticles without fullerenes.

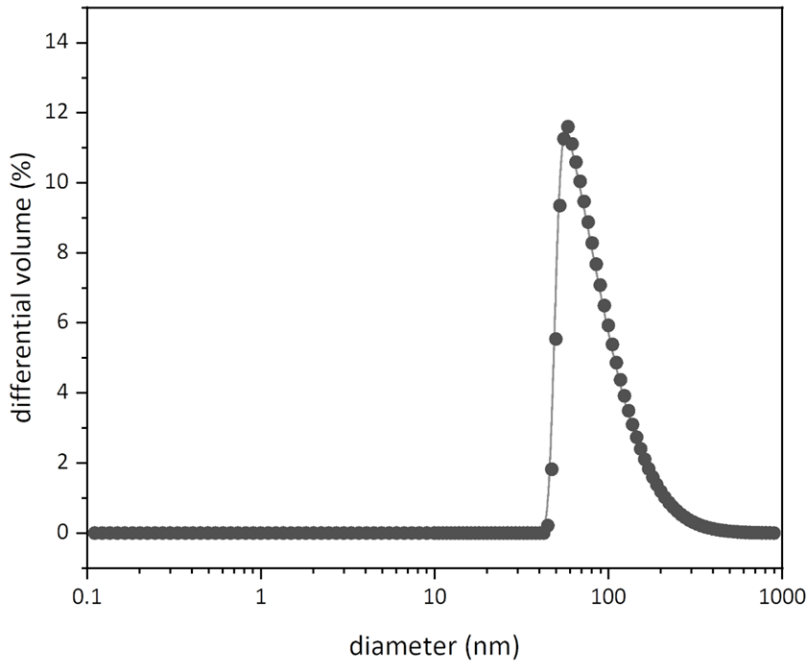

Figure S10. DLS data after freeze-drying and re-dispersing the core-crossliked polymer nanoparticles with fullerene C_{60}.

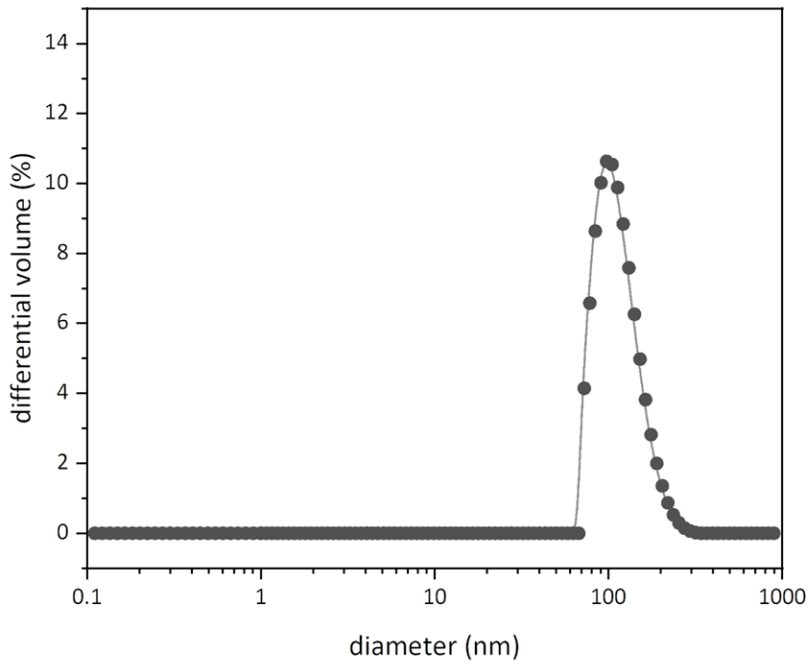

Figure S11. DLS data after freeze-drying and re-dispersing the core-crossliked polymer nanoparticles with fullerene C_{70}.

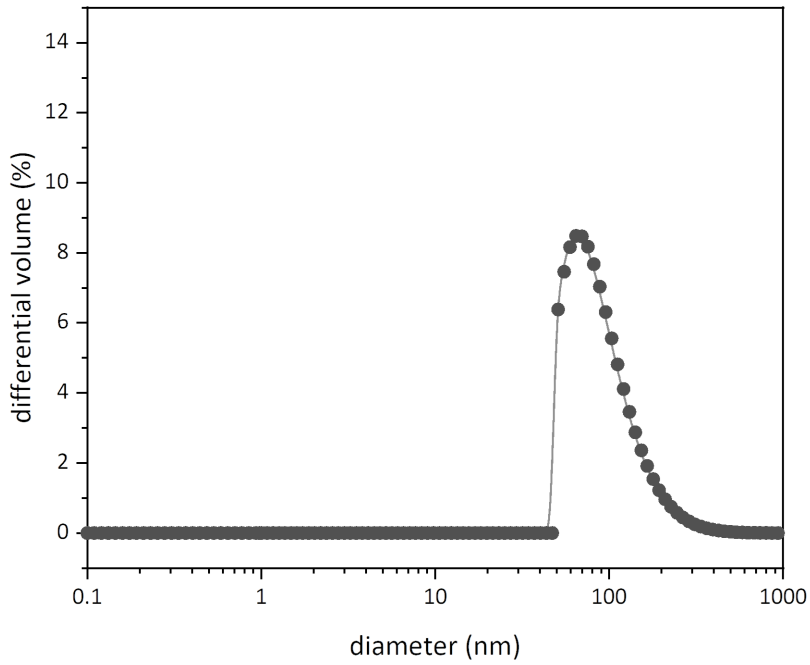

Figure S12. DLS data for the core-crosslinked polymer nanoparticles with fullerene C_{60} after storage for 6 months in water.

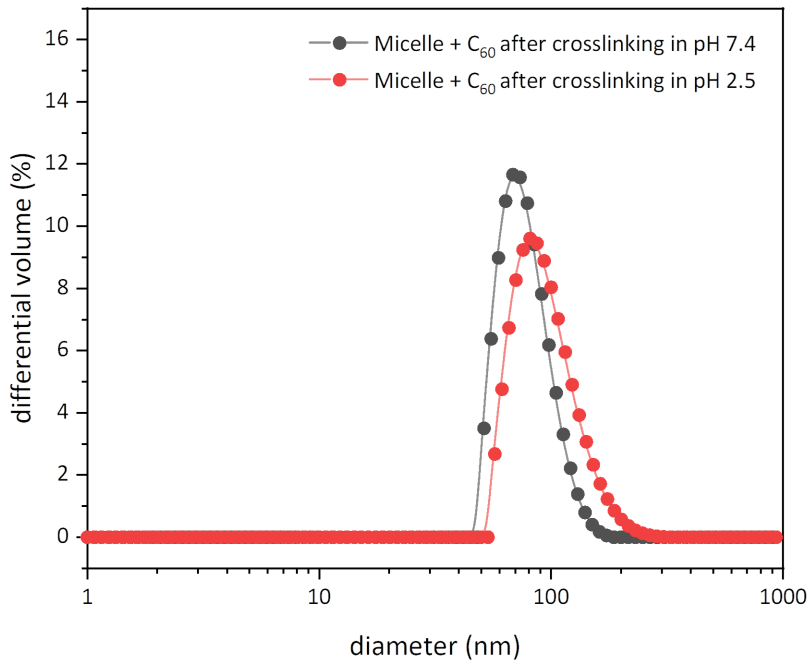

Figure S13. DLS data for the core-crosslinked polymer nanoparticles with fullerene C_{60} in pH 7.4 (PBS) and 2.5 (citric acid/Na_2HPO_4) buffer solutions.

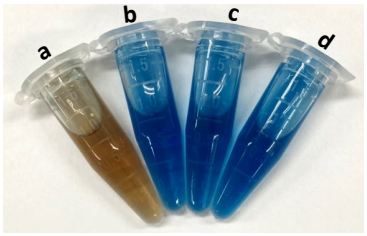

Figure S14. Digital picture showing Bradford reagent with (a) and without (b) BSA, and polymer nanoparticles containing C_{60} (c) and C_{70} (d).

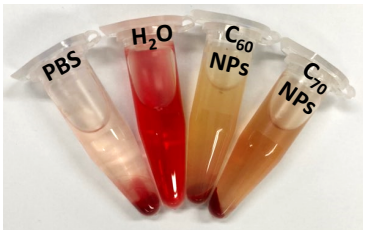

Figure S15. The negative control (PBS), positive control (DI water), and polymer nanoparticles (NPs) containing C_{60} and C_{70} in PBS with red blood cells.