Electronic Supplementary Information

Design of Fluorescently Tagged Poly(alkyl cyanoacrylate) Nanoparticles for Human Brain Endothelial Cells Imaging

Davide Brambilla,[1] Julien Nicolas,[1,] Benjamin Le Droumaguet,[1] Karine Andrieux,[1,*] Véronique Marsaud,[1] Pierre-Olivier Couraud,[2,3] Patrick Couvreur[1]*

[1]Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie, Université Paris-Sud, UMR CNRS 8612, Faculté de Pharmacie, 5 rue Jean-Baptiste Clément, F-92296 Châtenay-Malabry cedex, France.

[2]Institut Cochin, Université Paris Descartes, UMR CNRS 8104, 75014 Paris, France.

[3]Inserm, U567, Paris, France.

[*] To whom correspondence should be addressed:

Email: julien.nicolas@u-psud.fr Tel.: +33 1 46 83 58 53; Fax: +33 1 46 83 59 46

Email: karine.andrieux@u-psud.fr Tel.: +33 1 46 83 58 12; Fax: +33 1 46 83 59 46

Materials

Poly(ethylene glycol) monomethyl ether (MePEG, $M_{n,\ NMR}$ = 1910 g.mol^{-1}, $DP_{n,\ NMR}$ = 43, Fluka), cyanoacetic acid (99 %, Fluka), *N*,*N*′-dicyclohexylcarbodiimide (DCC, >99 %, Fluka), 4-dimethylaminopyridine (DMAP, 99 %, Aldrich), formaldehyde (37 % in water, Aldrich), pyrrolidine (99 %, Aldrich), anhydrous magnesium sulfate ($MgSO_4$, >99 %, Aldrich), triethyl amine (TEA, Aldrich) and dimethylsulfoxide (DMSO, 99.9 %, Bio Basic Inc.) were used as received. Rhodamine B alcohol[1] and dansyl alcohol[2] were synthesized as described elsewhere. 2-Propanol (99.5 %) and Pluronic F-68 (cell culture tested) was purchased from Fluka. All other solvents (tetrahydrofuran, (THF), methanol (MeOH), dichloromethane (DCM), diethyl ether (Et_2O), chloroform ($CHCl_3$), ethanol (EtOH), ethyl acetate (EtOAc) and hexane) were purchased at the highest grade from Carlo Erba. hCMEC/D3 human brain endothelial cell line was prepared as described elsewhere.[3-5] Dulbecco's phosphate buffer saline (DPBS) without $CaCl_2$ and $MgCl_2$ and EMB-2 medium were purchased from Lonza. Penicillin 10000 units-Streptomycin 10000 μg.mL^{-1} and Trypsin/EDTA were obtained from Invitrogen, Gibco. Hydrocortisone, human basic fibroblast growth factor (bFGF) cell culture tested and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 98 %) were purchased from Sigma. HEPES 1 M was purchased from PAA – The Cell Culture Company. Fetal bovine serum was purchased from Eurobio. Rat tail collagen type I was purchased from Becton-Dickinson. Fisher rat plasma was purchased from Charles River Laboratories.

Synthesis of hexadecyl cyanoacetate (HDCA)

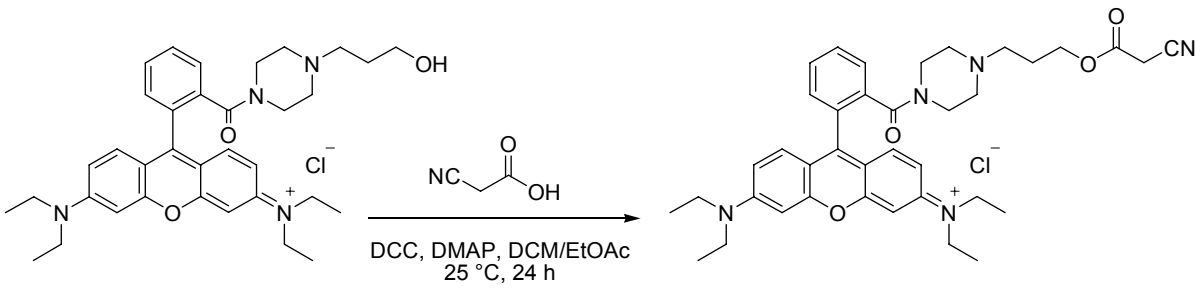
HDCA was synthesized as follows. In a 250 mL round bottom flask containing hexadecane-1-ol (10.65 g, 44×10^{-3} mol), cyanoacetic acid (7.48 g, 88×10^{-3} mol), EtOAc (5 mL) and DCM (50 mL) were introduced dropwise by a syringe over ca. 20 min, a solution of DCC (9.98 g, 48.4×10^{-3} mol) and DMAP (120 mg, 0.82×10^{-3} mol) in DCM (50 mL). The reaction medium was stirred during 24 h at ambient temperature under argon atmosphere. The solid was filtered off and the solvents were removed under reduced pressure. The solid was then purified by flash chromatography (SiO_2,

hexane/EtOAc; 5:1; v:v) to give a fine, white powder: 12.9 g (95 %). ^{1}H NMR δ = 0.88 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.14–1.50 (m, 26H, CH_2), 1.67 (m, J = 13.6, 6.8 Hz, 2H, $COOCH_2CH_2$), 3.45 (s, 2H, $CNCH_2$), 4.20 (t, J = 6.8 Hz, 2H, $COOCH_2CH_2$). IR (neat): υ (cm^{-1}) = 2261 (C≡N), 1728 (C=O).

Synthesis of methoxypoly(ethylene glycol) cyanoacetate (MePEGCA)

MePEGCA was synthesized as follows. In a 100 mL round bottom flask containing poly(ethylene glycol) monomethyl ether (11.0 g, DP_n = 45, 5.5×10^{-3} mol), cyanoacetic acid (0.955 g, 11.0×10^{-3} mol) and DCM (30 mL) were introduced dropwise by a syringe over ca. 20 min, a solution of DCC (2.27 g, 11.0×10^{-3} mol) and DMAP (60 mg, 0.41×10^{-3} mol) in DCM (10 mL). The reaction medium was stirred during 24 h at room temperature under argon atmosphere. The solid was filtered off and the solvent was removed under reduced pressure. The solid was then purified by recrystallization from isopropanol, filtered and dried under vacuum overnight to give a fine, white powder: 10.7 g (94 %). ^{1}H NMR δ = 3.34 (s, 3H, OCH_3), 3.53 (s, 2H, $CNCH_2$), 3.25–3.92 (m, 172H, OCH_2CH_2O), 4.32 (t, 2H, J = 4.5 Hz, $COOCH_2CH_2$). IR (neat): υ (cm^{-1}) = 1745 (C=O), 2251 (C≡N). $M_{n,\,SEC}$ = 1890 $g.mol^{-1}$, M_w/M_n = 1.04.

Synthesis of rhodamine B cyanoacetate (RCA)



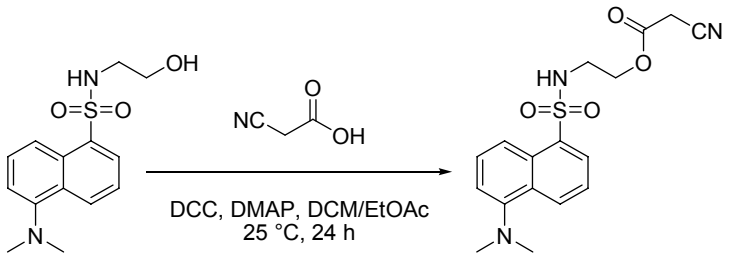
Scheme S1. Synthesis of rhodamine B cyanoacetate (RCA).

The rhodamine B cyanoacetate monomer was synthesized as follows. In a round bottom flask, rhodamine B alcohol[1] (450 mg, 0.74×10^{-3} mol) and cyanoacetic acid (127 mg, 1.49×10^{-3} mol)

This journal is (c) The Royal Society of Chemistry 2010

were dissolved in a mixture of DCM (10 mL) and EtOAc (1 mL). The resulting solution was bubbled for 30 min with N_2 while cooling down to 0 °C in an ice/water bath. To this solution was added dropwise over 20 min at 0 °C under N_2 a solution of DCC (169 mg, 0.82×10^{-3} mol) and DMAP (cat. amount) in DCM (10 mL). The reaction mixture was allowed to warm to room temperature and then stirred for 24 h. The resulting solution was filtered off to remove insoluble dicyclohexylurea and the precipitate was rinsed with dichloromethane until only a faint purple coloration was observed. The mother liquors were concentrated, redissolved in a minimum amount of DCM and precipitated in a large volume of cold diethyl ether. The precipitate was filtered and dried under high vacuum to give the pure product as purple crystals: 330 mg (70 % yield). ^{1}H NMR δ = 1.31 (t, 12H, *J* = 7.0 Hz, C*H*$_3$CH$_2$N), 2.17 (t, 2H, *J* = 6.2 Hz, CH$_2$C*H*$_2$CH$_2$), 3.20 (t, 2H, NC*H*$_2$(CH$_2$)$_2$O), 3.27 (bs, 4H, NCH$_2$C*H*$_2$NCH$_2$), 3.61 (q, 8H, *J* = 7.0 Hz, CH$_3$C*H*$_2$N), 3.70 (bs, 4H, C*H*$_2$N(C=O)), 3.72 (s, 2H, C*H*$_2$OCO), 4.25 (t, 2H, *J* = 6.0 Hz, C*H*$_2$CN), 6.72 (s, 2H, H$_{aromatic}$), 6.98 (d, 2H, *J* = 6.98 Hz, H$_{aromatic}$), 7.18 (d, 2H, *J* = 9.2 Hz, H$_{aromatic}$), 7.29 (d, 1H, *J* = 7.6 Hz, H$_{aromatic}$), 7.60–7.78 (m, 2H, H$_{aromatic}$). ^{13}C NMR δ = 10.28, 23.44, 25.08, 26.21, 46.09, 63.51, 96.15, 106.57, 113.62, 113.65, 114.28, 116.05, 128.35, 130.12, 130.45, 130.79, 131.76, 134.15, 155.59, 155.97, 157.64, 163.39, 166.58, 167.24. MS (+ESI) calculated for $C_{34}H_{46}N_5O_4^+$: 636.35; found: 636.5 ([M]$^{\bullet}$).

Synthesis of dansyl cyanoacetate (DCA)



Scheme S2. Synthesis of dansyl cyanoacetate (DCA).

The dansyl cyanoacetate monomer was synthesized as follows. In a round bottom flask, dansyl alcohol[2] (450 mg, 1.54×10^{-3} mol) and cyanoacetic acid (261 mg, 3.07×10^{-3} mol) were dissolved in

This journal is (c) The Royal Society of Chemistry 2010

a mixture of DCM (20 mL) and EtOAc (5 mL). The resulting solution was bubbled for 30 min with N_2 while cooling down to 0 °C in an ice/water bath. To this solution was added dropwise over 20 min at 0 °C under N_2 a solution of DCC (348 mg, 1.69×10^{-3} mol) and DMAP (cat. amount) in DCM (20 mL). The reaction mixture was allowed to warm to room temperature and then stirred for 24 h. The resulting solution was filtered off to remove insoluble dicyclohexylurea and the precipitate was rinsed with dichloromethane. The mother liquors were concentrated, redissolved in EtOAc and washed three times with water. The organic phase was dried over $MgSO_4$, filtered off and concentrated under vacuum. The crude product was purified on silica gel column eluting with a mixture hexanes/AcOEt (1:1) to afford the pure product as a yellow crystalline powder (360 mg, 65 % yield). ^{1}H NMR (DMSO-d_6, 298 K) δ = 2.88 (s, 6H, J = 7.0 Hz, $(CH_3)_2N$), 3.13 (q, 2H, J = 5.5 Hz, NHCH_2CH$_2$), 3.81 (s, 2H, CH_2CN), 4.06 (t, 2H, J = 5.5 Hz, CH_2OCO), 7.31 (d, 1H, J = 7.5 Hz, $H_{aromatic}$), 7.67 (q, 2H, J = 7.3 Hz, $H_{aromatic}$), 8.17 (d, 1H, J = 7.3 Hz, $H_{aromatic}$), 8.23 (t, 1H, J = 5.6 Hz, NH), 8.32 (d, 1H, J = 8.7 Hz, $H_{aromatic}$), 8.52 (d, 1H, J = 8.5 Hz, $H_{aromatic}$). ^{13}C NMR (DMSO-d_6, 298 K) δ = 24.31, 40.87, 45.02, 64.22, 114.77, 115.16, 118.98, 114.28, 123.59, 127.87, 128.25, 128.93, 129.06, 129.54, 135.77, 151.38, 164.08. MS (+ESI) calculated for $C_{17}H_{19}N_3O_4S$: 361.11; found: 384.20 ($[M+Na]^+$).

Synthesis of poly[hexadecyl cyanoacrylate-*co*-rhodamine B cyanoacrylate-*co*-methoxypoly(ethylene glycol) cyanoacrylate] (P(HDCA-*co*-RCA-*co*-MePEGCA)) fluorescent copolymer.

A typical synthesis of P(HDCA-*co*-RCA-*co*-MePEGCA) fluorescent copolymer (expt. **1**, Table 1) was as follows. In a 50 mL round bottom flask containing MePEGCA (0.4 g, 1.9×10^{-4} mol), HDCA (0.26 g, 8.4×10^{-4} mol), RCA (29.72 mg, 4.4×10^{-5} mol, 4.10 mol.% in the initial cyanoacetate mixture), EtOH (5 mL) and DCM (10 mL) under magnetic stirring, was sequentially introduced dropwise by a syringe over ca. 20 min, formaldehyde (0.4 mL, 5.3×10^{-3} mol) and pyrrolidine (20 µL, 2.4×10^{-4} mol). The mixture was allowed to stir during 24 h at room temperature and was then

This journal is (c) The Royal Society of Chemistry 2019

concentrated under reduced pressure. The residue was taken into DCM and washed multiple times with water. The resulting organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure and dried under vacuum to give a purple, waxy solid. The copolymer was analyzed by ^{1}H NMR and SEC. The same procedure was applied with different initial amounts of RCA (5.94 and 1.13 mg for expt **2** and **3**, respectively (see Table S1)).

Synthesis of poly[hexadecyl cyanoacrylate-*co*-dansyl cyanoacrylate-*co*-methoxypoly(ethylene glycol) cyanoacrylate] (P(HDCA-*co*-DCA-*co*-MePEGCA)) fluorescent copolymer.

In a 50 mL round bottom flask containing MePEGCA (0.4 g, 1.9×10^{-4} mol), HDCA (0.26 g, 8.4×10^{-4} mol), DCA (15.97 mg, 4.4×10^{-5} mol, 4.08 mol.% in the initial cyanoacetate mixture), EtOH (4 mL) and DCM (8 mL) under magnetic stirring, was sequentially introduced dropwise by a syringe over ca. 20 min, formaldehyde (0.4 mL, 5.3×10^{-3} mol) and pyrrolidine (20 µL, 2.4×10^{-4} mol). The mixture was allowed to stir during 24 h at room temperature and was then concentrated under reduced pressure. The residue was taken into DCM and washed multiple times with water, once with 1M HCl and once with brine. The resulting organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure and dried under vacuum to give a yellow, waxy solid. The copolymer **C4** was analyzed by ^{1}H NMR and SEC (see Table S2). ^{1}H NMR ($CDCl_3$, 298 K) δ = 0.87 (t, 16H, CH_2CH_3), 1.1–1.5 (bm, 120H, CH_2CH_3), 1.6–1.8 (bm, 10H, $COOCH_2CH_2CH_2$), 2.3–2.9 (bm, 10H, $C(CN)CH_2$), 3.37 (s, 3H, OCH_3), 3.4–3.9 (bm, 172H, CH_2CH_2O), 4.1–4.5 (bm, 10H, $COOCH_2CH_2$).

Preparation of nanoparticles

Nanoparticles were prepared by the nanoprecipitation technique.[6] In practice, the copolymer **C1** (20 mg) was dissolved in acetone (2 mL), and the copolymer solution was added dropwise to an aqueous solution 0.5 % (w/v) of Pluronic F68 (4 mL) under vigorous mechanical stirring. A milky suspension was observed almost instantaneously. Acetone was then evaporated under reduced pressure and nanoparticles were purified by ultracentrifugation (150 000 *g*, 1 h, 4 °C, Beckman Coulter, Inc.).

This journal is (c) The Royal Society of Chemistry 2010

The supernatant was discarded and the pellet was resuspended in the appropriated volume of water to yield a stable nanoparticles suspension (**N1**) of 5 $mg.mL^{-1}$. The same procedure was applied for copolymers **C2, C3** and **C4** to yield nanoparticle suspensions **N2**, **N3** and **N4** respectively.

Cytotoxicity of nanoparticles

The cytotoxicity of rhodamine B-tagged nanoparticles was investigated by MTT viability test on hCMEC/D3 human brain endothelial cell line. Cells were cultured according to previous studies.[3-5] Briefly, cells were grown on Type I collagen-coated plates in EBM-2 basal medium supplemented with fetal bovine serum 5 %, hydrocortisone 1.4 µM, basic fibroblast growth factor 1 $ng.mL^{-1}$, pen-strep 1 % and HEPES 10 mM. Polystyrene 96 wells plates were used and cells were seeded in each well (15 000 cells per well) with the medium previously described. The day after, an aqueous suspension of nanoparticles was administrated at three different concentrations: 10, 20 and 30 $\mu g.mL^{-1}$. After 48 h incubation at 37 °C and 5 % CO_2, the MTT reagent (at a final concentration of 0.05 % in DPBS) was administered and 3 h later, the percentage of living cells was evaluated with 96 wells plate absorbance reader at 570 nm. Cells treated with the same volume of water were used as negative controls. All these results were compared with non-fluorescent P(HDCA-*co*-MePEGCA) nanoparticles.

Plasma stability of nanoparticles

The fluorescent nanoparticles (**N2**) at 0.5 $mg.mL^{-1}$ or 0.3 $mg.mL^{-1}$ were incubated *in vitro* in Fischer rat plasma at 37 °C following a previously described protocol.[7] At predetermined time intervals (5 min, 30 min, 4 h and 8 h), an aliquot of the plasma medium was withdrawn and ultrafiltrated (Nanosep Centrifugal Devices 100 kDa, Pall Corporation) at 10 000 *g* during 20 min upon which the soluble degradation products were collected in the bottom chamber. Subsequently, the fluorescence intensity of the upper chamber (containing the nanoparticles) was measured by fluorescent spectroscopy and the results were expressed as a percentage of the initial measured fluorescence in the plasma medium.

This journal is (c) The Royal Society of Chemistry 2010

In vitro imaging with fluorescent nanoparticles

In vitro imaging was performed on hCMEC/D3 human brain endothelial cell line incubated with rhodamine B-tagged nanoparticles. Cells were seeded on type-I collagen-coated glass disk (25 mm in diameter) at a concentration of 25 000 cells.cm^{-2}. After 2 days, an aqueous suspension of nanoparticles (25 μg.mL^{-1}) was incubated with hCMEC/D3 cells. Following a 12 h incubation time, the cells monolayer was washed with fresh medium and then analyzed by confocal laser scanning microscopy.

Analytical techniques

^{1}H and ^{13}C NMR spectra were performed in deuterated chloroform ($CDCl_3$) or in dimethyl sulfoxide (DMSO) at ambient temperature on a Bruker Avance (300 MHz and 75 MHz, respectively). IR spectra were obtained on a Fourier Transform Bruker Vector 22 spectrometer. Size exclusion chromatography (SEC) was performed at 30 °C with two columns from Polymer Laboratories (PL-gel MIXED-D; 300 × 7.5 mm; bead diameter: 5 μm; linear part: 400 – 4 × 10^5 g.mol^{-1}) and a differential refractive index detector (Spectrasystem RI-150 from Thermo Electron Corp.). The eluent was chloroform ($CHCl_3$) at a flow rate of 1 mL.min^{-1} and toluene was used as a flow-rate marker. The calibration curve was based on poly(methyl methacrylate) (PMMA) standards (peak molar masses, M_p = 625 – 625 500 g.mol^{-1}) from Polymer Laboratories. This technique allowed M_n (the number-average molar mass), M_w (the weight-average molar mass) and M_w/M_n (the polydispersity index, PDI) to be determined. Nanoparticles diameter (D_z) was measured by dynamic light scattering (DLS) with a Nano ZS from Malvern (173° scattering angle) at a temperature of 25 °C. The particle size distribution is generally considered as narrow when below 0.10. The surface charge of nanoparticles was investigated by ζ-potential (mV) measurement at 25 °C after dilution with NaCl 1 mM and using the Smoluchowski equation. DLS and ζ-potential measurements were used to study nanoparticles stability as a function of time at 37 °C. Fluorescence spectroscopy (Perkin-Elmer LS50B) with 10 mm optical quartz cuvette (Hellma 101-QS Suprasil) was used to evaluate the fluorescence properties of RCA, DCA, P(HDCA-*co*-RCA-*co*-MePEGCA) and

This journal is (c) The Royal Society of Chemistry 2010

P(HDCA-*co*-DCA-*co*-MePEGCA) copolymers as well as associated nanoparticles. Maximum emission ($\lambda_{ex.}$) and maximum excitation ($\lambda_{em.}$) wavelengths were determined as well as the maximum intensity of fluorescence (in a.u.) at different concentrations. The same procedure was followed for nanoparticles (**N1**, **N2**, **N3** and **N4**) in aqueous solution. *In vitro* imaging experiments were performed with a confocal laser scanning microscope LSM 510 META (Zeiss, Germany) equipped with a 1 mW Helium Neon laser and a Plan-Apochromat 63X objective lens (Numerical Aperture / 1.4, oil immersion). Fluorescence was collected with long-pass 560 nm emission filter under 543 nm wavelength excitation. To specifically consider intracellular nanoparticles localization, acquisitions were made at the median plane of the cell monolayer. The pinhole size was set at 1.0 Airy unit (106 µm diameter) giving an optical section thickness of 0.8 µm. Prior to observations, it was checked that autofluorescence of hCMEC/D3 cells was negligible under the acquisition settings and did not interfere with the fluorescence coming from the nanoparticles.

Characterization of fluorescently tagged copolymers and nanoparticles

Table S1. Synthesis of Poly[hexadecyl cyanoacrylate-*co*-rhodamine B cyanoacrylate-*co*-methoxypoly(ethylene glycol) cyanoacrylate] (P(HDCA-*co*-RCA-*co*-MePEGCA) Copolymers (C) and Associated Nanoparticles (N).

Expt.	RCA	M_n[b]	M_w/M_n[b]	Average particle diameter (D_z)	Particle size distribution[c]	Zeta potential (ζ)
	mol.%[a]	g.mol^{-1}		nm		mV
1 (C1, N1)	4.10	1370	1.96	115 ± 7.3	0.118	–40.6 ± 0.3
2 (C2, N2)	0.85	1330	1.94	124 ± 9.9	0.106	–35.0 ± 3.4
3 (C3, N3)	0.16	1400	1.99	123 ± 5.5	0.078	–30.9 ± 3.2

[a]molar fraction in the initial cyanoacetate mixture. [b]determined by SEC with a calibration curve based on PMMA standards. [c]given by the DLS apparatus.

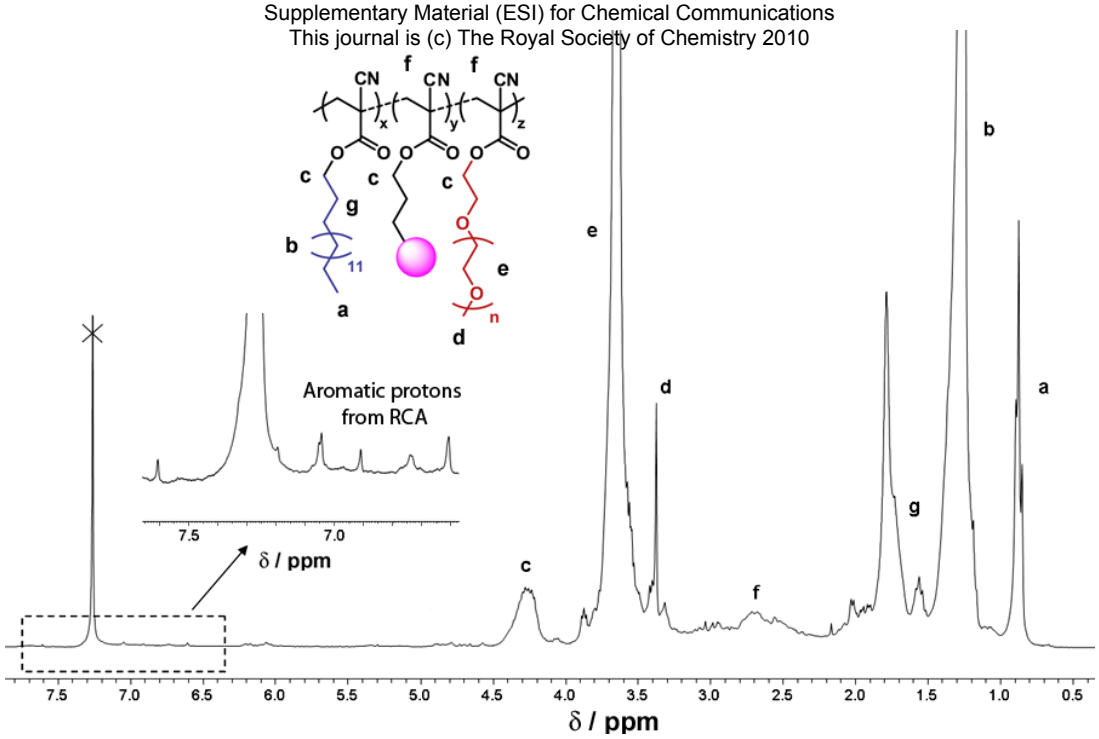


Figure S1 300 MHz ^{1}H NMR of the fluorescent P(HDCA-*co*-RCA-*co*-MePEGCA) copolymer **C1** in $CDCl_3$. Insert: enlarged area in the 6.6–7.6 ppm region. Quantification of RCA in the copolymers was not undertaken due to its very low percentage, which would have led to strong inaccuracies.

Table S2 Synthesis of Poly[hexadecyl cyanoacrylate-*co*-dansyl cyanoacrylate-*co*-methoxypoly(ethylene glycol) cyanoacrylate] (P(HDCA-*co*-DCA-*co*-MePEGCA) Copolymers (**C4**) and Associated Nanoparticles (**N4**).

Expt.	RCA	M_n[b]	M_w/M_n[b]	Average particle diameter (D_z)	Particle size distribution[c]	Zeta potential (ζ)
	mol.%[a]	g.mol^{-1}		nm		mV
4 (C4, N4)	4.07	1640	2.27	89 ± 0.5	0.172	–31.6 ± 0.6

[a]molar fraction in the initial cyanoacetate mixture. [b]determined by SEC with a calibration curve based on PMMA standards. [c]given by the DLS apparatus.

This journal is (c) The Royal Society of Chemistry 2010

Colloidal characteristics of fluorescently tagged poly(alkyl cyanoacrylate) nanoparticles

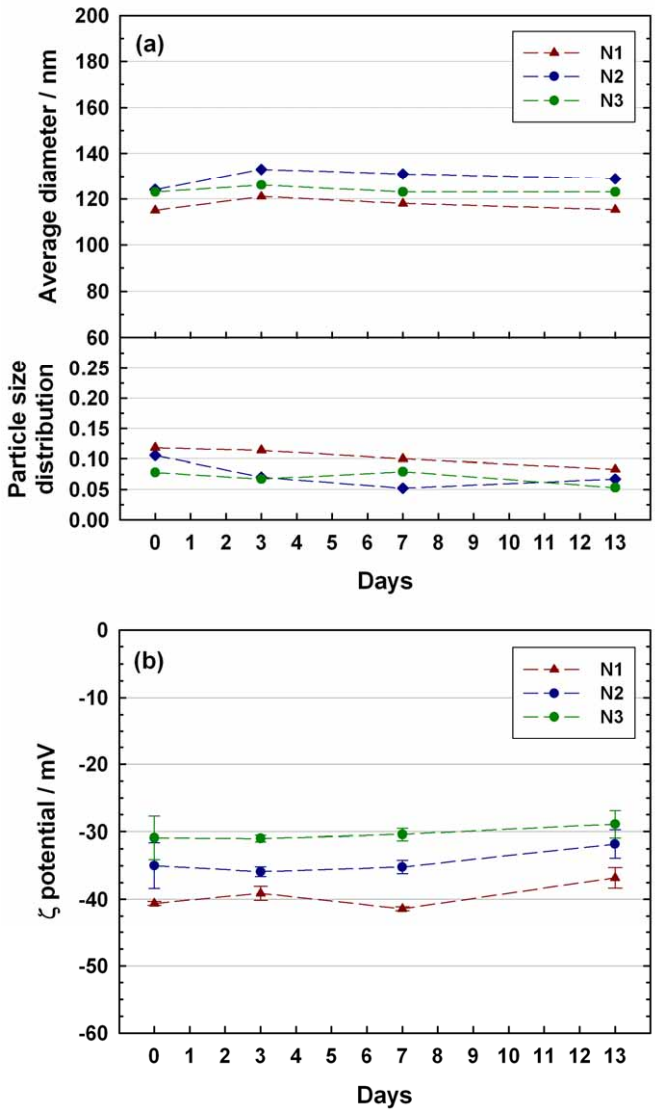


Figure S2. Evolution of average diameters and particle size distribution (a) and ζ-potential values (b) of P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticles (**N1**, **N2** and **N3**) in water at 37 °C as a function of time.

Fluorescent properties of rhodamine B-tagged poly(alkyl cyanoacrylate) copolymers and nanoparticles

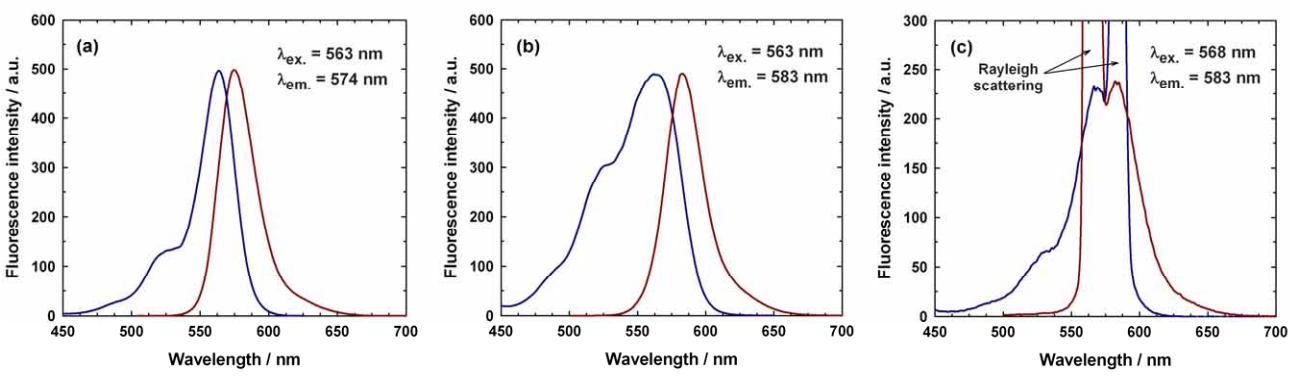


Figure S3. Normalized excitation (blue line) and emission (red line) spectra of rhodamine B cyanoacetate (RCA) in $CHCl_3$ (a), P(HDCA-*co*-RCA-*co*-MePEGCA) copolymer **C2** in $CHCl_3$ (b) and P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticles **N2** in water (c).

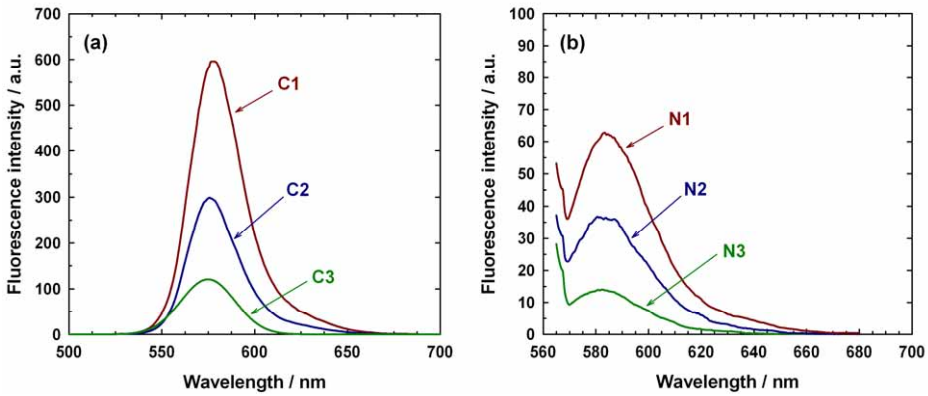


Figure S4. Fluorescence emission spectra of P(HDCA-*co*-RCA-*co*-MePEGCA) copolymer solutions in $CHCl_3$ at 0.1 $mg.mL^{-1}$ (a) and of P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticle suspensions in water at 0.1 $mg.mL^{-1}$ (b).

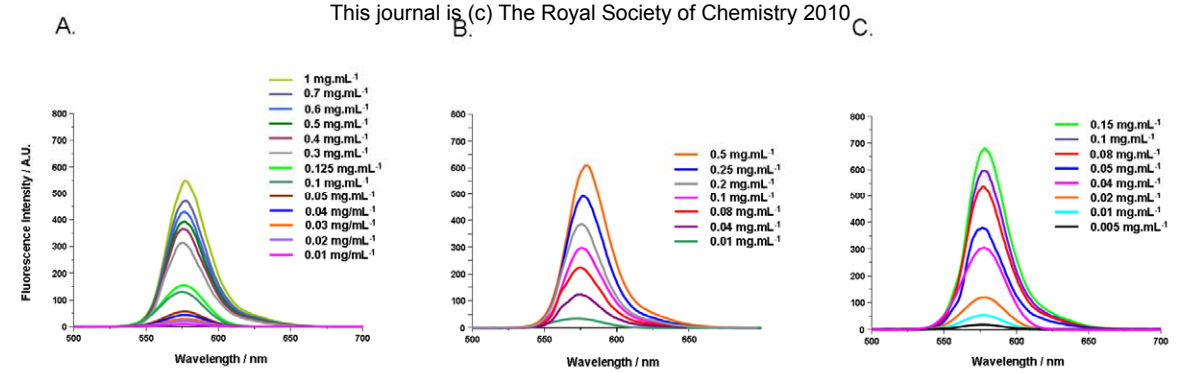


Figure S5. Emission spectra in $CHCl_3$ of P(HDCA-*co*-RCA-*co*-MePEGCA) copolymer **C3** (a), **C2** (b) and **C1** (c) as the function of the copolymer concentration.

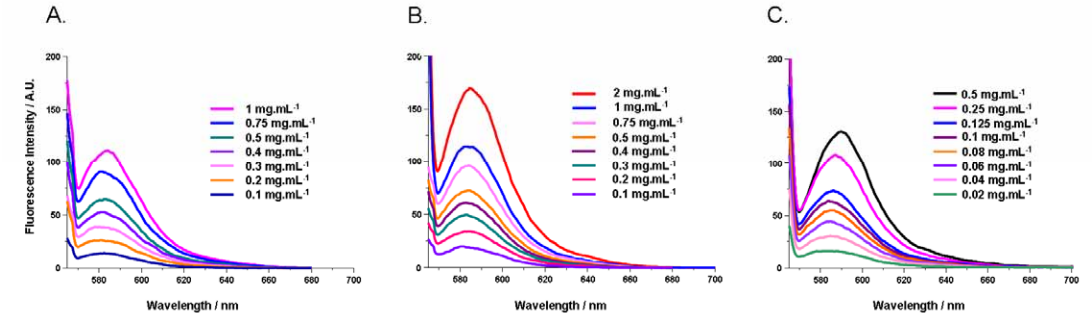


Figure S6. Emission spectra in water of P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticle suspensions **N3** (a), **N2** (b) and **N1** (c) as the function of the nanoparticle concentration.

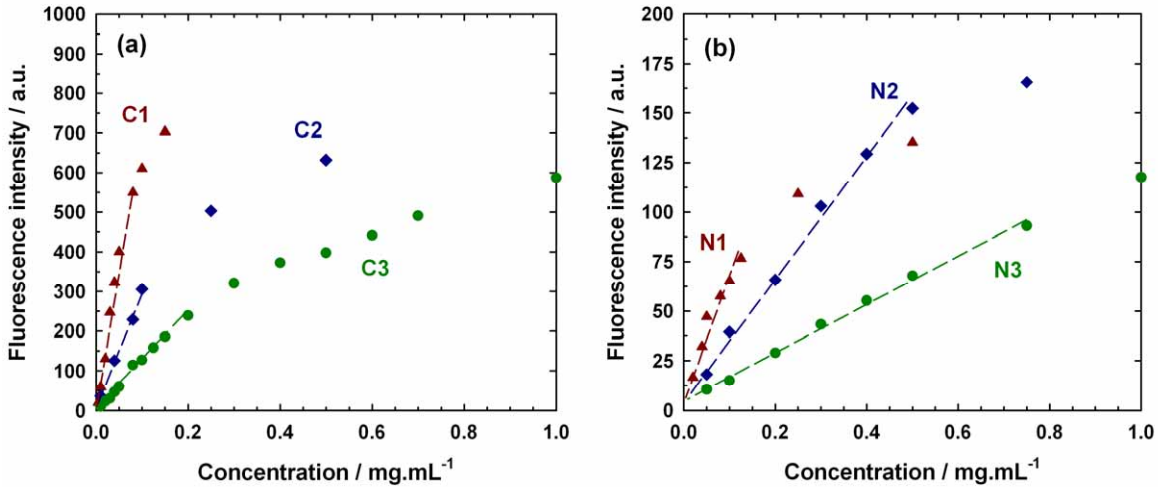


Figure S7. Evolution of fluorescence intensity as a function of P(HDCA-*co*-RCA-*co*-MePEGCA) copolymer concentration (a) and P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticles concentration (b).

Fluorescent properties of dansyl-tagged poly(alkyl cyanoacrylate) copolymers and nanoparticles

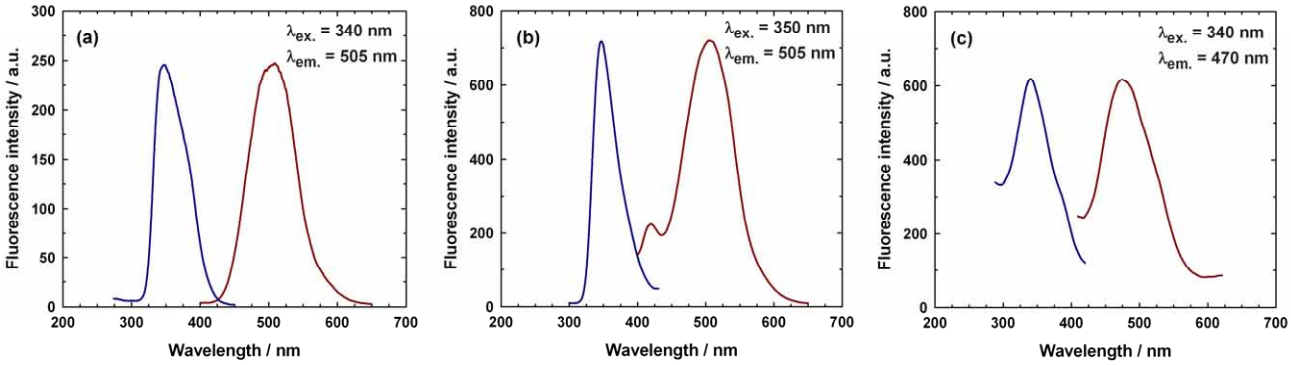


Figure S8. Normalized excitation (blue line) and emission (red line) spectra of dansyl cyanoacetate (DCA) in acetone (a), P(HDCA-*co*-DCA-*co*-MePEGCA) copolymer **C4** in acetone (b) and P(HDCA-*co*-DCA-*co*-MePEGCA) nanoparticles **N4** in water (c).

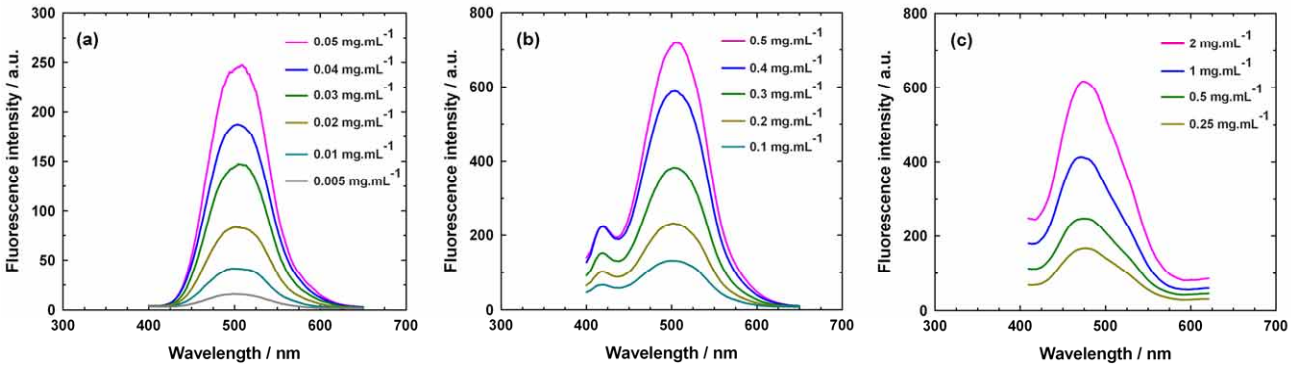


Figure S9. Emission spectra of dansyl cyanoacetate (DCA) in acetone (a), P(HDCA-*co*-DCA-*co*-MePEGCA) copolymer **C4** in acetone (b) and P(HDCA-*co*-DCA-*co*-MePEGCA) nanoparticles **N4** in water (c) as the function of the concentration.

Application to *in vitro* cell imaging

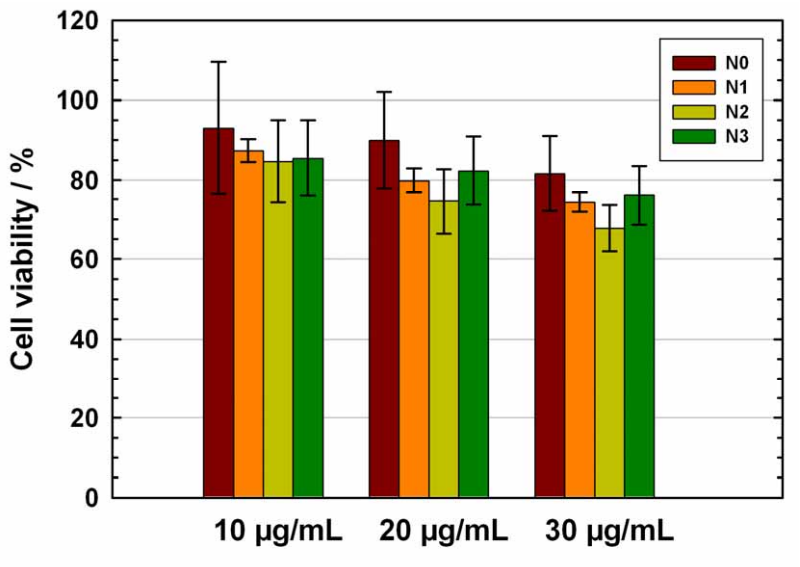


Figure S10. Cell viability (MTT assay) after 48 h incubation of hCMEC/D3 human brain endothelial cell line with non-fluorescent P(HDCA-*co*-MePEGCA) (**N0**) or fluorescent P(HDCA-*co*-RCA-*co*-MePEGCA) (**N1**, **N2**, **N3**) nanoparticles as a function of nanoparticles concentration.

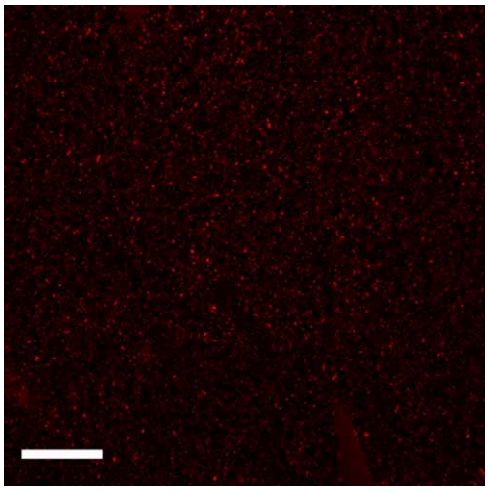


Figure S11. Confocal microscopy images of fluorescently tagged nanoparticles (**N1**). Scale bar = 25 µm; Laser illumination: 543 nm; detection gain: 494; laser power: 100 %.

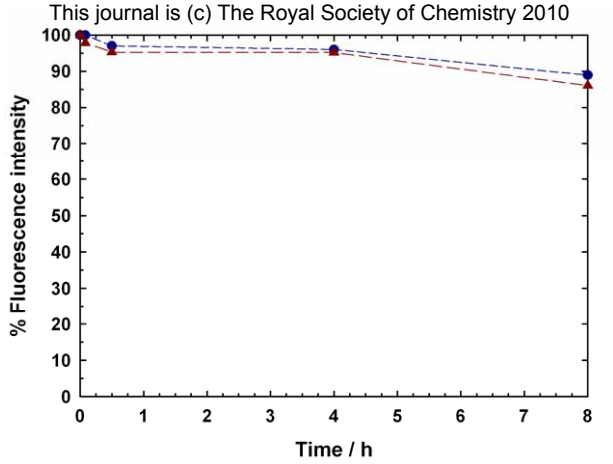


Figure S12. In vitro biodegradation profile of fluorescent P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticles (**N2**) at 0.5 mg.mL^{-1} (●) or 0.3 mg.mL^{-1} (▲) in Fischer rat plasma at 37 °C.

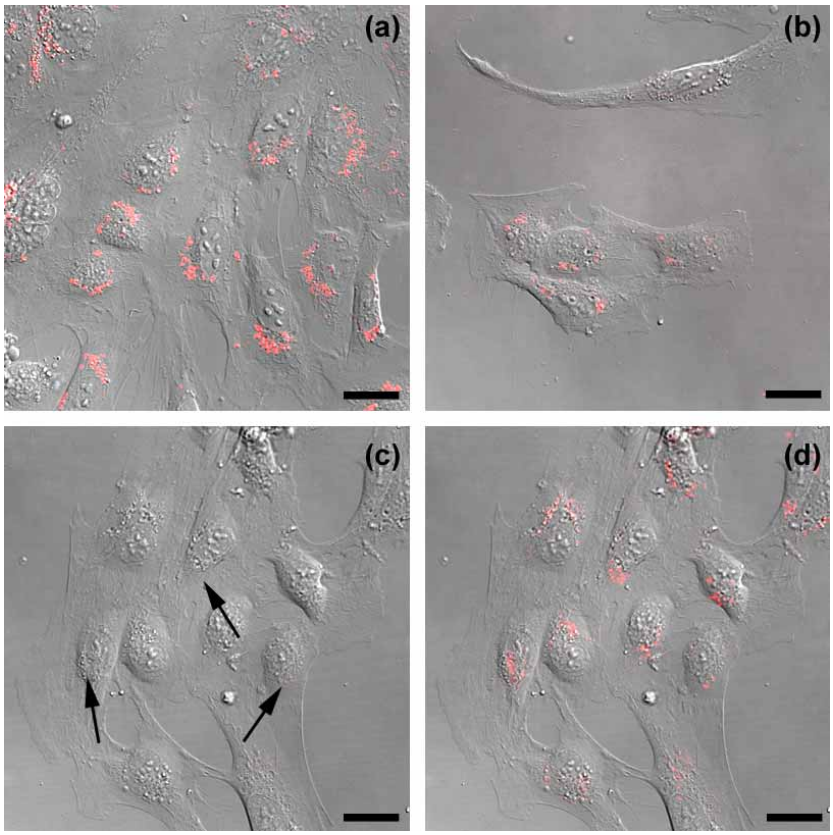


Figure S13. Fluorescence images (red) superimposed with hCMEC/D3 human brain endothelial cells Nomarski images viewed from the top cell surface after incubation with fluorescent P(HDCA-*co*-RCA-*co*-MePEGCA) nanoparticles for 12 h and subsequent washing of the medium. (a): **N1**; (b): **N2**; (c): **N3** and (d) **N3** with increased gain and laser power. The arrows indicate faint fluorescence areas. Scale bars = 20 μm.

This journal is (c) The Royal Society of Chemistry 2010

1. T. Nguyen and M. B. Francis, *Org. Lett.*, 2003, **5**, 3245.
2. T. W. Kim, J.-h. Park and J.-I. Hong, *J. Chem. Soc., Perkin Trans. 2*, 2002, 923.
3. G. Schreibelt, G. Kooij, A. Reijerkerk, R. van Doorn, S. I. Gringhuis, S. van der Pol, B. B. Weksler, I. A. Romero, P. O. Couraud, J. Piontek, I. E. Blasig, C. D. Dijkstra, E. Ronken and H. E. de Vries, *FASEB J.*, 2007, **21**, 3666.
4. B. B. Weksler, E. A. Subileau, N. Perriere, P. Charneau, K. Holloway, M. Leveque, H. Tricoire-Leignel, A. Nicotra, S. Bourdoulous, P. Turowski, D. K. Male, F. Roux, J. Greenwood, I. A. Romero and P. O. Couraud, *FASEB J.*, 2005, **19**, 1872.
5. L. Cucullo, P. O. Couraud, B. Weksler, I. A. Romero, M. Hossain, E. Rapp and D. Janigro, *J. Cereb. Blood. Flow Metab.*, 2008, **28**, 312.
6. O. Thioune, H. Fessi, J. P. Devissaguet and F. Puisieux, *Int. J. Pharm.*, 1997, **146**, 233.
7. I. Brigger, J. Morizet, G. Aubert, H. Chacun, M. J. Terrier-Lacombe, P. Couvreur and G. Vassal, *J. Pharmacol. Exp. Ther.*, 2002, **303**, 928.