Heterofunctional Polymers and Core-Shell Nanoparticles via Cascade Aminolysis/Michael Addition and Alkyne-Azide Click Reaction of RAFT Polymers

Zesheng An,*[a] Wei Tang, [b] Minghong Wu,[a] Zheng Jiao,[a] and Galen D. Stucky*[b]

[a] *Institute of Nanochemistry and Nanobiology, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, P. R. China. Email: an.zesheng@shu.edu.cn*.

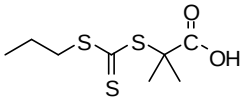
[b]*Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA. Email:stucky@chem.ucsb.edu.*

Supporting Information

General

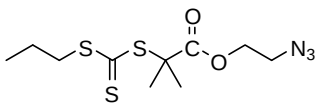
All polymers and nanoparticles were synthesized under microwave conditions in sealed reaction tubes using a Biotage microwave (2.45 GHz, Initiator Eight). NMR spectra were collected on a Varian Unity Inova 400 MHz NMR Spectrometer using deuterated chloroform as solvent and its residue (7.24 ppm) as reference. Absorption spectra of polymer/nanoparticle solutions were collected on a Shimazu UV-1800 spectrometer. All UV-Vis measurements were performed on three samples and were reported as the average. Molecular weights and polydispersity indices of polymers were characterised with gel permeation chromatography (GPC) using DMF (containing 0.01wt% LiBr) as the eluting solvent. GPC measurements were performed with a Waters 2690 pump equipped with a Waters 2414 Differential Refractometer. Separation was effected by Waters Styragel HR columns (0.5, 2, 4 and 5) at a flow rate of 1 ml/min. Molecular weights and polydispersity indices of polymers were evaluated with the Waters Empower software using narrowly dispersed PMMA as standard. For molecular weight determination by UV-Vis, the absorption of trithiocarbonate group (ε = 14 300)[1] at 310 nm was used. The hydrodynamic diameters of nanoparticles were determined by dynamic light scattering (DLS) technique on a Zetasizer Nano-ZS (Malvern Instrument) using a 633 nm laser and the scattered light

was collected at 173º. The as-prepared colloidal solutions were diluted with Millipore water until the size was no longer concentration dependant and a well-defined correlation curve was obtained. All measurements were performed at 25 ± 0.1 ºC. Z-average diameter and polydispersity were automatically analysed in the cumulant mode by the Malvern Zetasizer software and were reported as the average of three measurements.



2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid

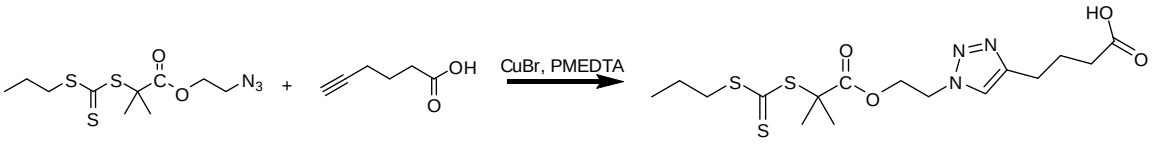
The chain transfer agent was synthesized according to Lai's method[2]: A solution of 7.6 g propane thiol, 48.2 g acetone and 1 mol% dodecyltrimethylammonium bromide as phase transfer agent was cooled at 0 °C. 17 g 50 wt% NaOH solution was added dropwise, followed by the addition of 7.6 g carbon disulfide in 10.2 g acetone. The solution was stirred for 1 hour before the addition of 17.9 g chloroform, followed by the addition of 71.6 g 50 wt% NaOH solution dropwise. The reaction was stirred at 0 °C for 2 hours and then at room temperature overnight. The reaction was acidified to pH < 2 with concentrated hydrochloric acid, extracted with ethyl ether (50 ml) three times. The ether solution was dried over magnesium sulfate before removal of solvent. The yellow oily residue was purified by chromatography eluting with 10% (v:v) ethyl acetate/hexane. 11.2 g yellow oil was obtained (73.5%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.84 (broad s, 1H), 3.25 (t, J = 7.4 Hz, 2H), 1.69 (m, 8H), 0.99 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 220.9, 179.7, 55.8, 39.0, 25.4, 25.3, 21.6, 13.7. HRMS (EI): calculated for C8H14O2S3, 238.0156; found, 238.0161.



2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester

A solution of 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (1.0 g, 4.2 mmol) and 2-azidoethanol (0.60 g, 5.04 mmol) in 10 ml methylene chloride was cooled to 0 °C, to which EDC hydrochloride (0.96 g, 5.0 mmol) and 4-dimethylaminopyridine (0.1 g, 0.82 mmol) were added successively. The reaction was stirred at 0 °C for 1 hour and then at room temperature overnight. The reaction was washed with water (15 ml) twice, 1.5 M HCl solution (15 ml) twice and then water (15 ml) twice. The organic solution was dried over magnesium sulfate and the solvent was removed. The yellow oily residue was purified by chromatography eluting with 5% (v:v) ethyl acetate/hexane. 1.0 g yellow oil was obtained (77.4%).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 4.25 (t, J = 5.0 Hz, 2H), 3.44 (t, J = 5.0 Hz, 2H), 3.25 (t, J = 5.0 Hz, 2H), 1.70 (m, 8H), 0.99 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 221.9, 173.0, 64.8, 55.9, 49.8, 38.9, 25.4, 24.3, 21.6, 13.6. HRMS(ESI/TOF): calculated for C10H17N3O2S3, 307.0483; found for M+Na (C10H17N3O2S3Na), 330.0396.



Click reaction between 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester and 5-hexynoic acid.

Cu(I)Br (4.6 mg, 32.1 μmol) was placed in an argon degassed tube, to which a degassed solution containing 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester (0.1 g, 0.325 mmol), 5-hexynoic acid (41.4 mg, 0.33 mmol) and *N*,*N*,*N'*,*N''*,*N'''*-pentamethyldiethylenetriamine (PMEDTA) (5.6 mg, 32.5 μmol) in 2 ml DMF was added. The reaction tube was sealed with a septum and degassed with argon for 30 min. During the reaction, the solution gradually turned into green. A solution of sodium ascorbate (11.8 mg, 59.4 μmol) in 0.3 ml degassed water was added to reduce the oxidized catalyst. After the reaction was stirred at room temperature overnight, TLC test showed

complete disappearance of 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester. The product was purified by chromatography eluting with a gradient of solvent starting with ether and ending with 10% ethanol/ether solution. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.39 (s, 1H), 4.56 (t, J = 5.2 Hz, 2H), 4.45 (t, J = 5.2 Hz, 2H), 3.21 (t, J =7.4 Hz, 2H), 2.77 (t, J =7.4 Hz, 2H), 2.41 (t, J =7.4 Hz, 2H), 1.20 (t, J =7.4 Hz, 2H), 1.7-1.6 (m, 8H), 0.97 (t, J =7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 222.0, 178.0, 172.7, 147.4, 122.8, 121.5.64.0, 55.8, 49.2, 39.0, 33.4, 25.5, 25.3, 24.8, 21.6, 13.7. HRMS(ESI/TOF): calculated for C16H25N3O4S3: 419.1007; found for M+H C16H26N3O4S3, 420.1093.

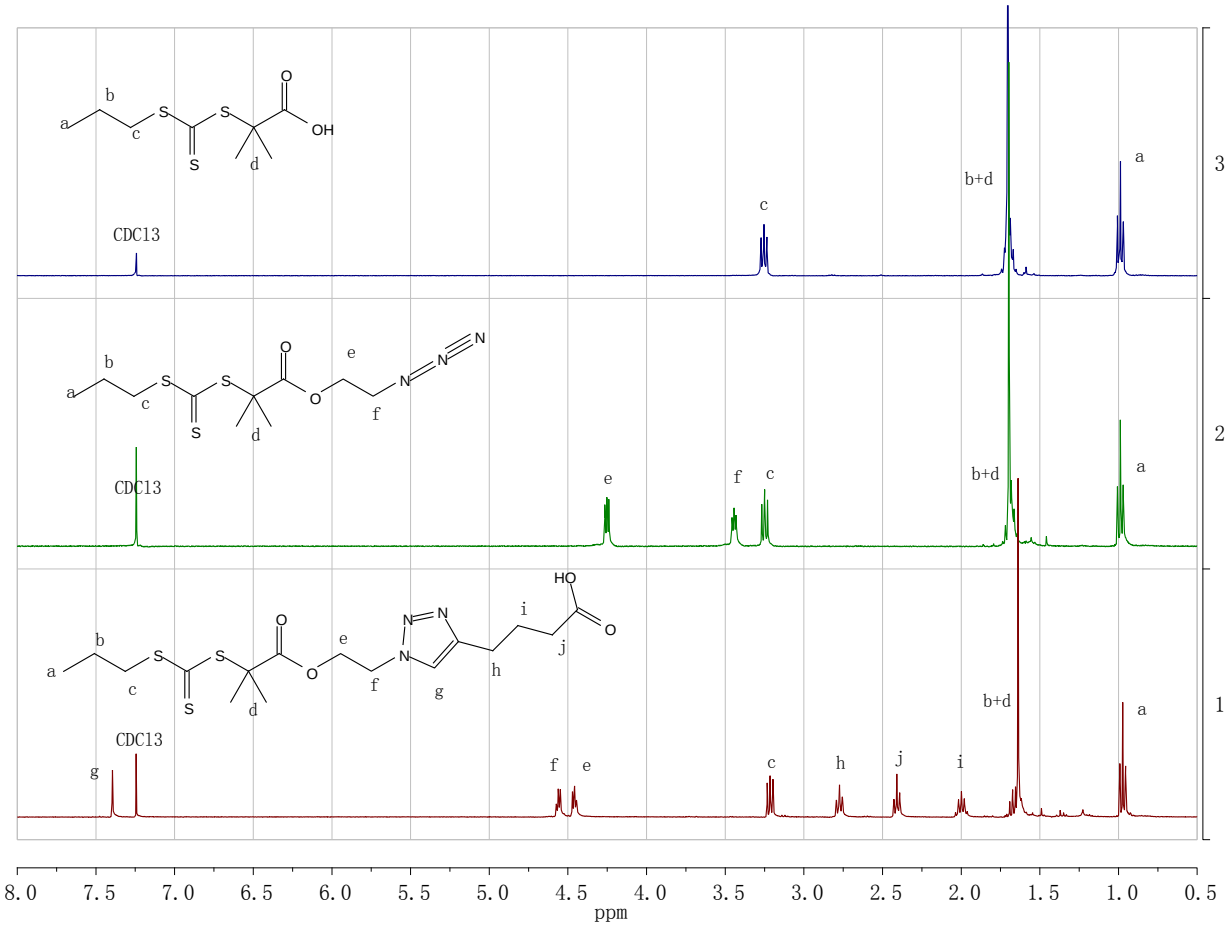
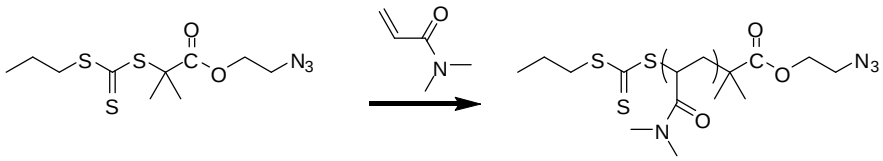


Fig.S1 ^{1}H NMR spectra of 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (top), 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester (middle) and the

product of click reaction between 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid and 2-propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester (bottom).

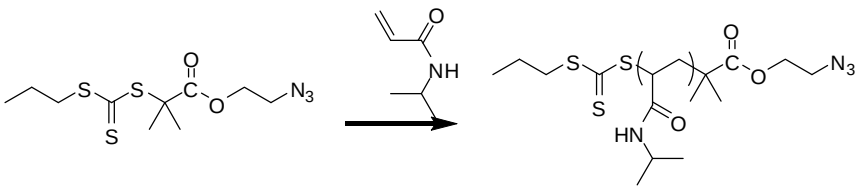
Preparation of RAFT polymers.

All polymers were prepared using AIBN as initiator in dioxane under microwave irradiation conditions at 70 °C for desired amount of time.



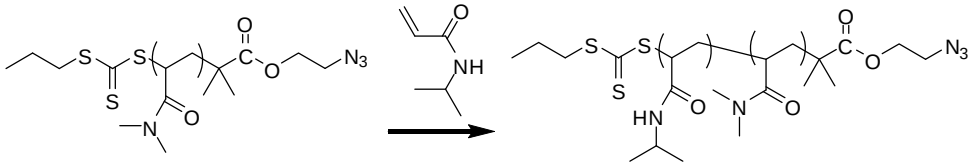
Examplary procedure for the preparation of azido-functionalised poly(*N,N'*-dimethylacrylamide).

2-Propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester (76.8 mg, 0.25 mmol), *N,N'*-dimethylacrylamide (2.5 g, 25.0 mmol) and AIBN (4.1 mg, 0.025 mmol) were dissolved in 10 ml dioxane in a capped microwave vial. The solution was degassed via a syringe with argon for 40 min. The degassed solution was subject to microwave irradiation at 70 °C for 70 min. The solution was precipitated into 150 ml diethyl ether and the precipitate was collected by filtration. The collected polymer was dissolved in 15 ml THF and was precipitated into 150 ml diethyl ether. The precipitation process was performed twice. 2.07 g slightly yellow solid was obtained at 80% yield. The collected polymer was dried under vacuum at 60 °C overnight for characterisations. M_n = 12 980, M_w/M_n = 1.09.



Examplary procedure for the preparation of azido-functionalised poly(*N*-isopropylacrylamide).

2-Propylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid 2-azidoethyl ester (65.9 mg, 0.21 mmol), *N*-isopropylacrylamide (2.4 g, 21.4 mmol) and AIBN (3.6 mg, 0.021 mmol) were dissolved in 10 ml dioxane in a capped microwave vial. The solution was degassed via a syringe with argon for 60 min. The degassed solution was subject to microwave irradiation at 70 °C for 70 min. The solution was precipitated into 150 ml diethyl ether and the precipitate was collected by filtration. The collected polymer was dissolved in 15 ml THF and was precipitated into 150 ml diethyl ether. The precipitation process was performed twice. 1.8 g slightly yellow solid was obtained at 70% yield. The collected polymer was dried under vacuum at 60 °C overnight for characterisations. M_n = 11 780, M_w/M_n = 1.07.



Examplary procedure for the preparation of block copolymers through chain extension of PDMA

Macromolecular chain transfer agent, azido-functionalized poly(*N,N'*-dimethylacrylamide) (M_n = 6 900, M_w/M_n = 1.14) (0.15 g, 0.0217 mmol), *N*-isopropylacrylamide (0.38 g, 3.255 mmol) and AIBN (0.7 mg, 4.34 μmol) were dissolved in 4 ml dioxane. After the solution was degassed with argon for 1 h, it was subject to microwave irradiation at 70 °C for 70 min. The solution was then precipitated into 100 ml diethyl ether, and the precipitate was collected through filtration. The collected solid was dissolved in 10 ml THF and was precipitated into 100 ml diethyl ether. The precipitation process was performed twice. 0.38 g slightly yellow solid was collected at 71.7% yield. The collected polymer was dried under vacuum at 60 °C overnight for characterisations. M_n = 18 200, M_w/M_n = 1.10.

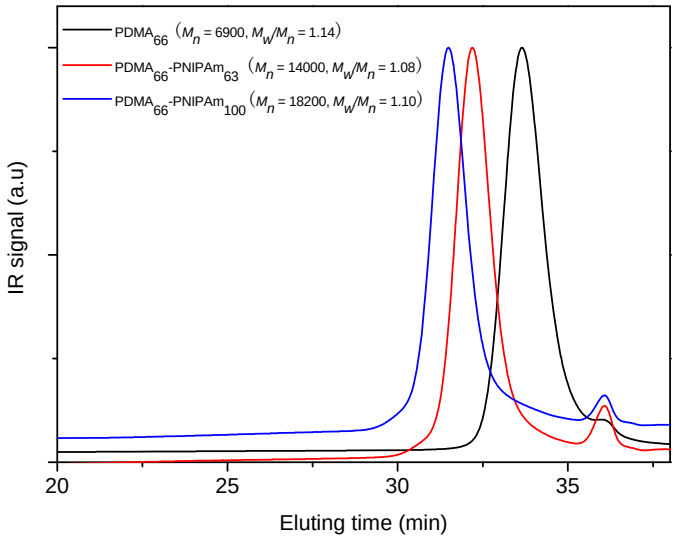
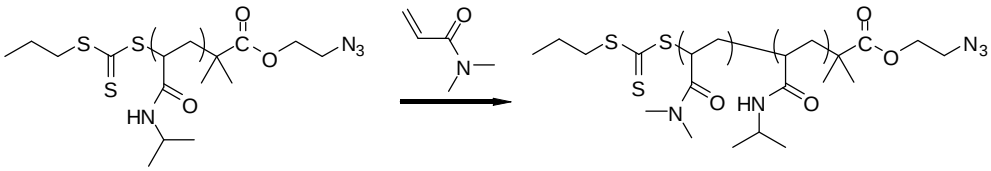


Fig.S2 GPC traces of a PDMA homopolymer and two PDMA-PNIPAm block copolymers synthesized via chain extension of PDMA.



Examplary procedure for the preparation of block copolymers through chain extension of PNIPAm

Macromolecular chain transfer agent, azido-functionalised poly(*N*-isopropylacrylamide) (M_n = 11 780, M_w/M_n = 1.07) (0.2 g, 0.017 mmol), *N*,*N*'-dimethylacrylamide (0.106 g, 1.06 mmol) and AIBN (0.6 mg, 3.4 μmol) were dissolved in 2 ml dioxane. After the solution was degassed with argon for 1 h, it was subject to microwave irradiation at 70 °C for 70 min. The solution was then precipitated into 50 ml diethyl ether, and the precipitate was collected through filtration. The collected solid was dissolved in 5 ml THF and was precipitated into 50 ml diethyl ether. The precipitation process was performed twice.

0.2 g slightly yellow solid was collected at 65% yield. The collected polymer was dried under vacuum at 60 °C overnight for characterisations. M_n = 17 500, M_w/M_n = 1.09.

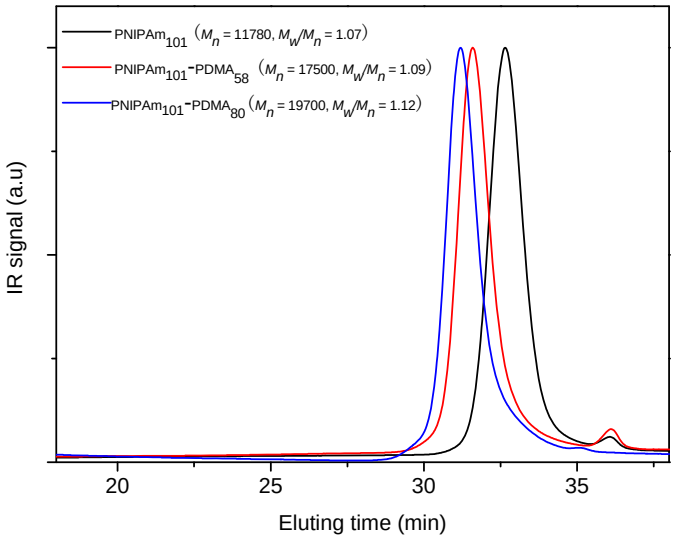
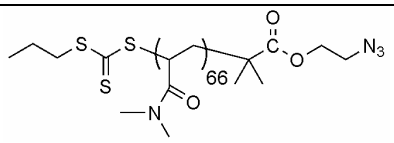
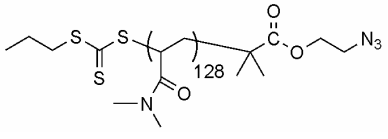
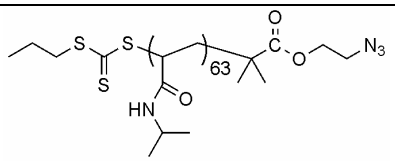
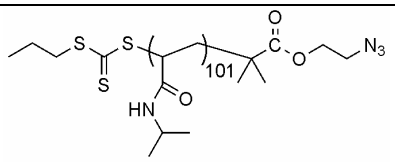
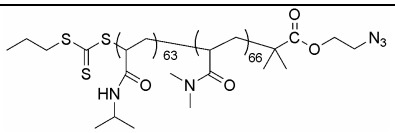
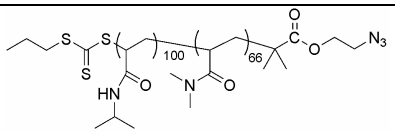
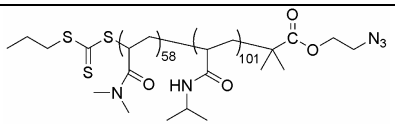
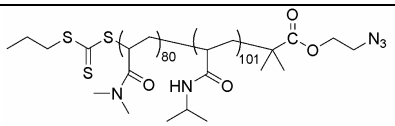


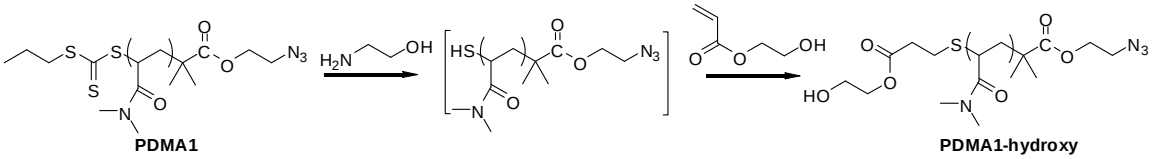
Fig.S3 GPC traces of a PNIPAm homopolymer and two PNIPAm-PDMA block copolymers synthesized via chain extension of PNIPAm.

Table S1. Structures and parameters of RAFT homopolymers and block copolymers.

Name	Structure	M_n (GPC)	M_n (UV-Vis)	M_w/M_n (GPC)
PDMA1, $PDMA_{66}$		6 900	5 850	1.14
PDMA2, $PDMA_{128}$		12 980	8 900	1.09

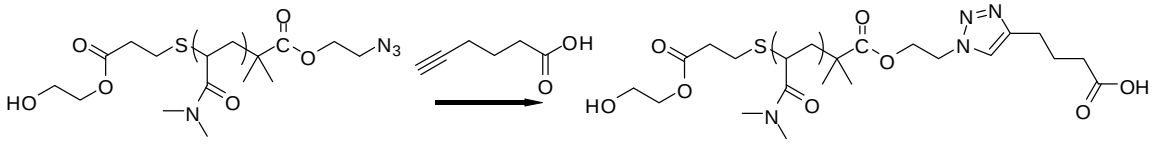
PNIPAm1, $PNIPAm_{63}$		7 400	6 870	1.12
PNIPAm2, $PNIPAm_{101}$		11 780	8 810	1.07
(PDMA-PNIPAm)1, $PDMA_{66}$-$PNIPAm_{63}$		14 000	10 960	1.08
(PDMA-PNIPAm)2, $PDMA_{66}$-$PNIPAm_{100}$		18 200	14 820	1.10
(PNIPAm-PDMA)1, $PNIPAm_{101}$-$PDMA_{58}$		17 500	13 030	1.09
(PNIPAm1-PDMA)2 $PNIPAm_{101}$-$PDMA_{80}$		19 700	19 270	1.12

Heterofunctional polymers synthesized by modification of polymers through one-pot aminolysis-Michael addition process and subsequent click chemistry



The azido-functionalised **PDMA1** (M_n = 6 900, M_w/M_n = 1.14) (0.6 g, 0.087 mmol) was dissolved in 6 ml THF in a septum-sealed tube. After the solution was degassed with argon for 40 min, 3 drops of degassed trioctylphosphine, 2-hydroxyethylamine (26.6 mg, 0.435 mmol) and 2-hydroxyethylacrylate (0.106 g, 0.87 mmol) were added successively via a syringe. After the reaction was stirred under argon

for 8 h, it was filtered via 0.2 μm filter into 150 ml diethyl ether. The precipitate was collected, dissolved in 5 ml THF and filtered into 100 ml diethyl ether. The precipitate was collected and dried under vacuum at 60 °C overnight. 0.36 g **PDMA1-hydroxy** was obtained at 60% yield. For characterisations, 95 mg of the obtained polymer was further purified by dialysis (MWCO 3 500) against frequently changed water for 2 days. M_n = 7 960, M_w/M_n = 1.10.



The **PDMA1-hydroxy** (M_n = 7 960, M_w/M_n = 1.10) (0.15 g, 0.022 mmol), prepared through one-pot aminolysis and Michael addition process, and 5-hexynoic acid (15.0 mg, 0.11 mmol) were dissolved in 3 ml water, to which $CuSO_4 \bullet 5H_2O$ (1.1 mg, 4.4 μmol) was added. The resulting solution was degassed with argon for 40 min, to which degassed sodium ascorbate (1.7 mg, 8.8 μmol) aqueous solution (0.2 ml) was injected via a syringe. After the reaction was stirred under argon at room temperature overnight, another bath of catalyst system was injected and the reaction was further stirred for 6 h. The polymer was purified by dialysis (MWCO 3 500) against frequently changed water for 3 days. In order to facilitate the removal of Cu catalyst, the use of low concentration (~ 2 mM) of EDTA was desired. The dialysed solution was filtered via 0.2 μm filter and the polymer **PDMA1-hydroxy-acid** was isolated by freeze-drying. 0.1 g solid was obtained at 66.6% yield. M_n = 8 670, M_w/M_n = 1.13.

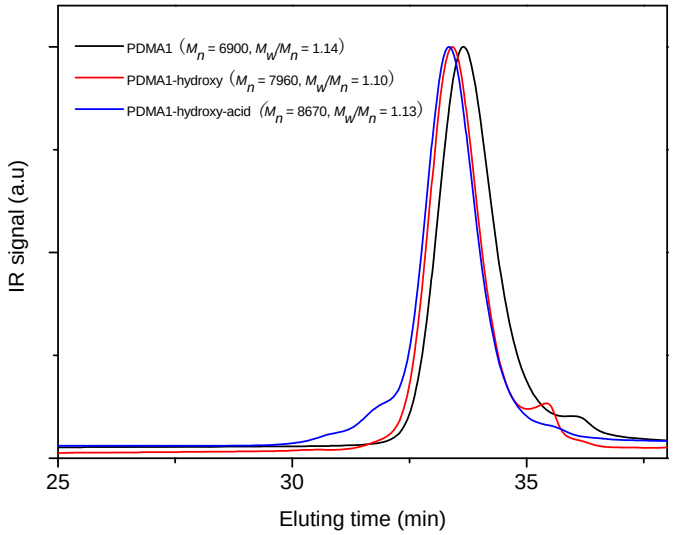


Fig.S4 GPC traces of PDMA, PDMA after one-pot aminolysis-Michael addition (PDMA-hydroxy) and PDMA after cascade aminolysis/Michael addition and click chemistry (PDMA-hydroxy-acid).

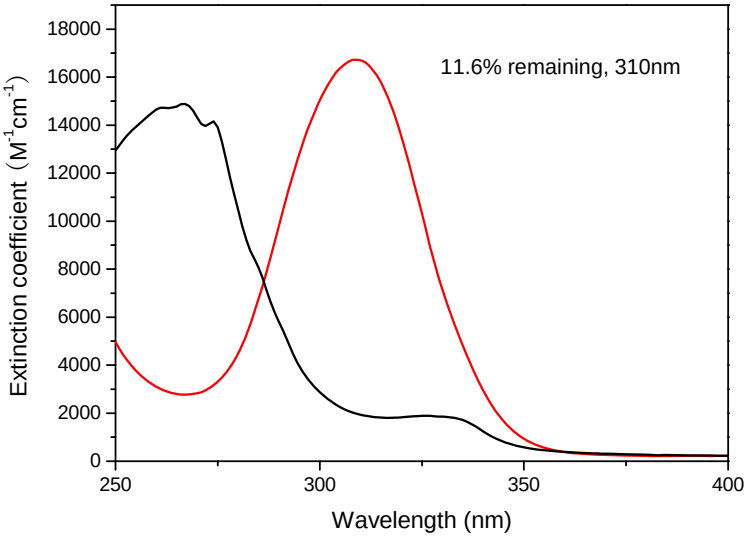
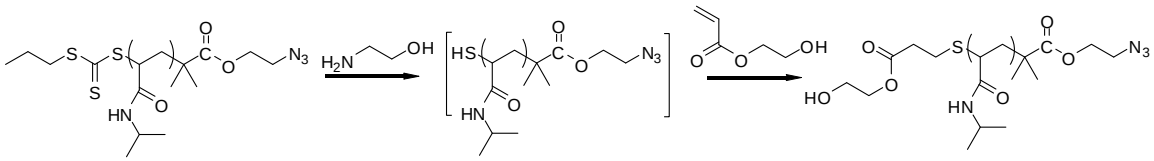
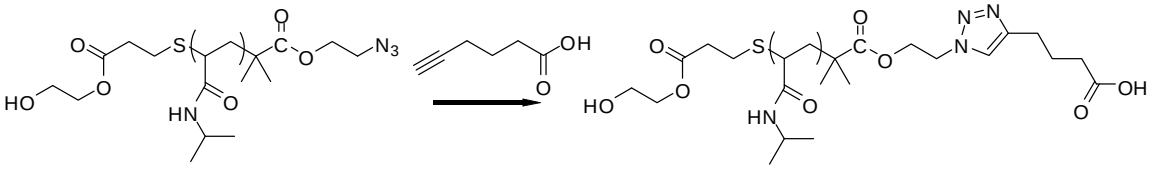


Fig.S5 UV-Vis absorption spectra of chloroform solutions of PDMA, PDMA after one-pot aminolysis/Michael addition (PDMA-hydroxy).



PNIPAm1 **PNIPAm1-hydroxy**

The azido-functionalised **PNIPAm1** (M_n = 7 400, M_w/M_n = 1.12) (0.6 g, 0.081 mmol) was dissolved in 4 ml THF in a septum-sealed tube. After the solution was degassed with argon for 40 min, 2 drops of degassed trioctylphosphine, 2-hydroxyethylamine (25.0 mg, 0.405 mmol) and 2-hydroxyethylacrylate (97.0 mg, 0.83 mmol) were added successively via a syringe. After the reaction was stirred under argon for 10 h, it was precipitated into 100 ml diethyl ether. The precipitate was collected via filtration, dissolved in 5 ml THF, and filtered via 0.2 μm filter into 100 ml diethyl ether. The precipitate was collected and dried under vacuum at 60 °C overnight. 0.53 g **PNIPAm1-hydroxy** was obtained at 88% yield. For characterisations, 0.1 g of the obtained polymer was further purified by dialysis (MWCO 3 500) against frequently changed water for 3 days. M_n = 8 800, M_w/M_n = 1.07.



PNIPAm1-hydroxy **PNIPAm1-hydroxy-acid**

The **PNIPAm1-hydroxy** (M_n = 8 800, M_w/M_n = 1.07) (0.2 g, 0.027 mmol), modified through one-pot aminolysis/Michael addition process, and 5-hexynoic acid (17.7 mg, 0.135 mmol) were dissolved in 6.8 ml water and 1 ml DMF, to which $CuSO_4 \bullet 5H_2O$ (1.4 mg, 5.4 μmol) was added. The resulting solution was degassed with argon for 40 min, to which degassed sodium ascorbate (2.1 mg, 10.8 μmol) aqueous solution (0.2 ml) was injected via a syringe. After the reaction was stirred under argon at room temperature overnight, the polymer was purified by dialysis (MWCO 3 500) against frequently changed water for 3 days. In order to facilitate the removal of Cu catalyst, the use of low concentration (~ 2 mM) of EDTA was desired. The dialysed solution was filtered via a 0.2 μm filter and the polymer

PNIPAm1-hydroxy-acid was isolated by freeze-drying. 0.15 g solid was obtained at 75% yield. M_n = 13 000, M_w/M_n = 1.15.

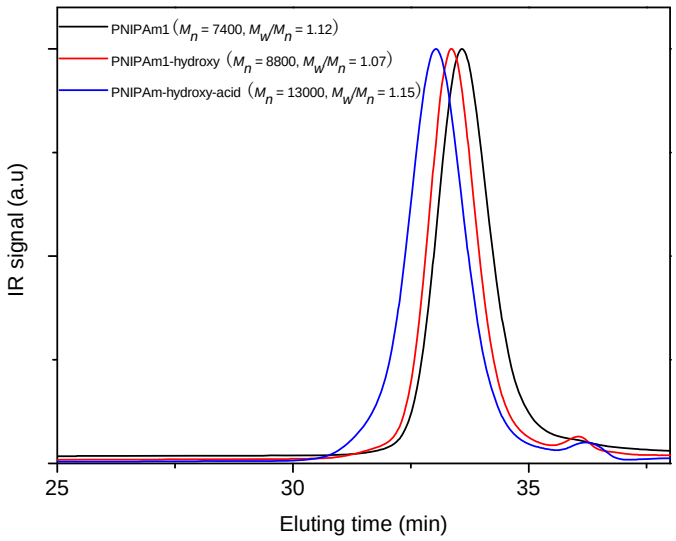


Fig.S6 GPC traces of PNIPAm, PNIPAm after one-pot aminolysis/Michael addition (PNIPAm-hydroxy) and PNIPAm after cascade aminolysis/Michael addition and click chemistry (PNIPAm-hydroxy-acid).

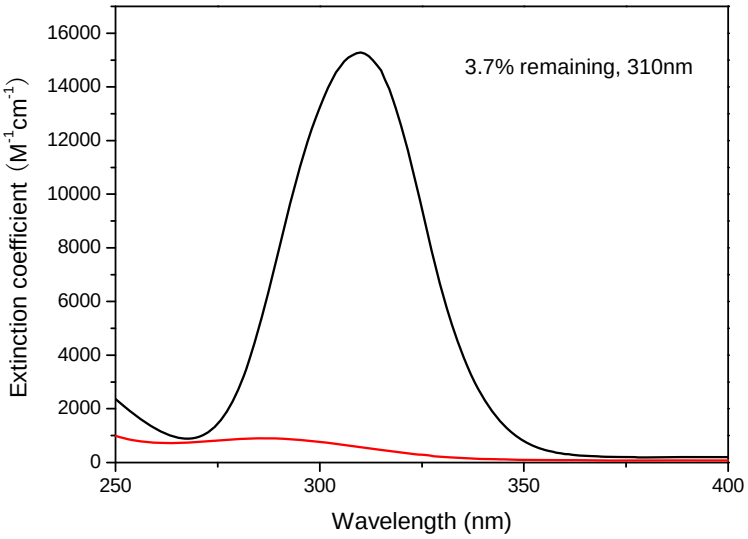


Fig.S7 UV-Vis absorption spectra of chloroform solutions of PNIPAm, PNIPAm after one-pot aminolysis/Michael addition (PNIPAm-hydroxy).

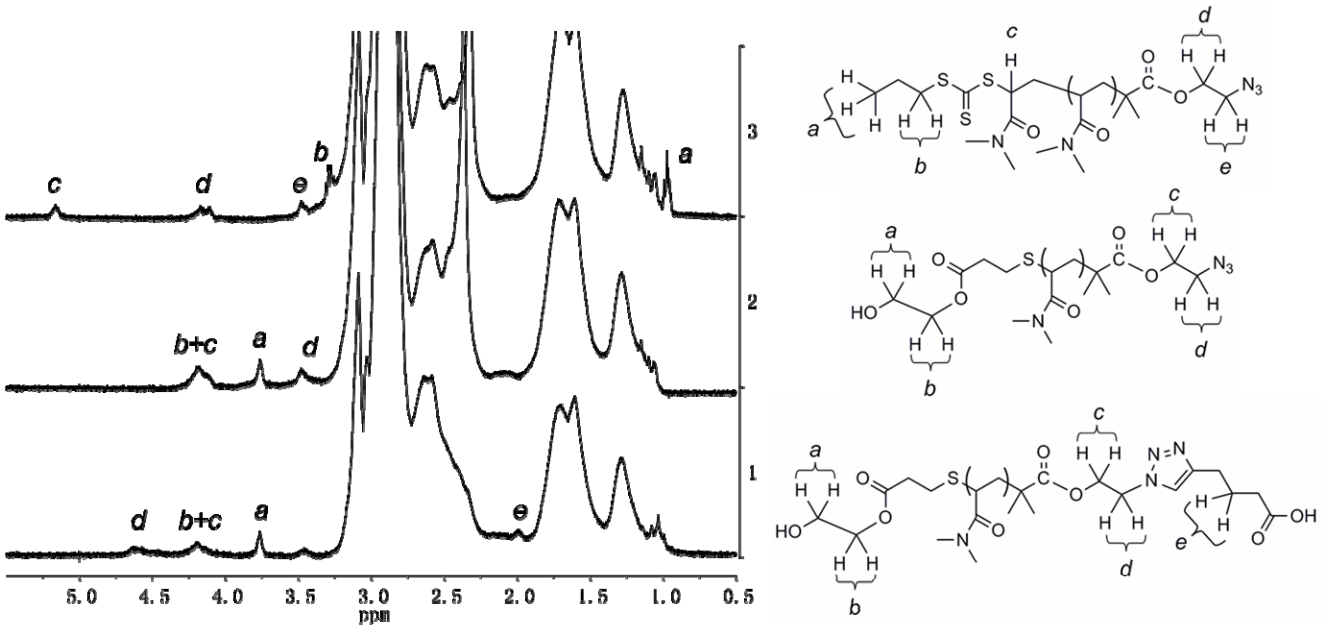


Fig.S8 ^{1}H NMR spectra of PDMA (top), PDMA-hydroxy (middle) and PDMA-hydroxy-acid (bottom).

Preparation of core-shell nanoparticles through RAFT-mediated precipitation polymerisation process under microwave conditions.

Core-shell nanoparticles were prepared according to our previously developed method[4] using azido-functionalised PDMA, synthesized via RAFT process, as both chain transfer agent and particle stabilising agent. In an examplary synthesis, macromolecular chain transfer agent PDMA2 (M_n = 12 980, M_w/M_n = 1.09) (82.6 mg, 6.4 μmol), *N*-isopropylacrylamide (0.15 g, 1.27 mmol), *N,N'*-methylenebisacrylamide (4.0 mg, 23.4 μmol) and 2,2'-azobis(2-methylpropionamidine)dihydrochloride (0.57 mg, 2.1 μmol) were dissolved in 4 ml water. The resulting solution was sealed in a microwave reaction tube and degassed with argon for 1 h. The degassed solution was subject to microwave irradiation at 70 °C for 1 h. The prepared core-shell nanoparticles had a diameter of 65 ± 1 nm with polydispersity of 6.5 ± 2.6% as determined by DLS.

Procedure for functionalisation of nanoparticles with fluorescein *o*-acrylate via one-pot aminolysis/Michael addition process

A portion of the synthesized nanoparticle solution (diameter 65 ± 1 nm, containing 2.1 μmol trithiocarbonate groups) was mixed with 0.1 ml 20 wt% sodium bisulfite solution. After the resulting solution was degassed with argon for 1 h, 0.1 ml degassed 2-hydroxyethylamine solution (1.3 mg, 21.0 μmol) was injected, followed by the injection of 0.2 ml degassed fluorescein *o*-acrylate DMF solution (8.2 mg, 21.0 μmol). The reaction was stirred under argon at room temperature overnight. The functionalised nanoparticle solution was purified via dialysis (MWCO 3 500) against frequently changed 20-40% ethanol solution for 2 days, and then frequently changed water for 2 days until UV-Vis measurements showed no absorption of fluorescein. The purified fluorescein-modified nanoparticle solution was designated as **NP-fluorescein**. DLS measurements showed that the particle size did not change before and after modification. UV-Vis measurements showed 36% of the

trithiocarbonate groups were functionalised with fluorescein (see procedure for quantification of fluorescein in **NP-fluorescein** below)

Procedure for functionalisation of nanoparticles (NP-fluorescein) with dansyl probe via click chemistry process

A portion of **NP-fluorescein** solution (containing 1.05μmol azido group) was combined with 1 ml dansyl probe[3] DMF solution (3.0 mg, 10.5 μmol) and 0.1 ml 10 mg/ml $CuSO_4 \bullet 5H_2O$ solution (1.0 mg, 4.2 μmol). After the resulting solution was degassed with argon for 1 h, 56 μl degassed sodium ascorbate solution (0.8 mg, 4.2 μmol) was injected. The reaction was stirred under argon at room temperature overnight. The modified nanoparticle solution was purified via dialysis (MWCO 3 500) against frequently changed 20-40% ethanol solution for 2 days, and then frequently changed water for 2 days until UV-Vis measurements showed no absorption of dansyl probe. The purified nanoparticle solution was designated as **NP-fluorescein-dansyl**. DLS measurements showed essentially no size change after the click chemistry process. UV-Vis measurements showed that the yield of functionalisation of **NP-fluorescein** with dansyl probe was essentially quantitative (see procedure for quantification of dansyl probe in **NP-fluorescein-dansy**).

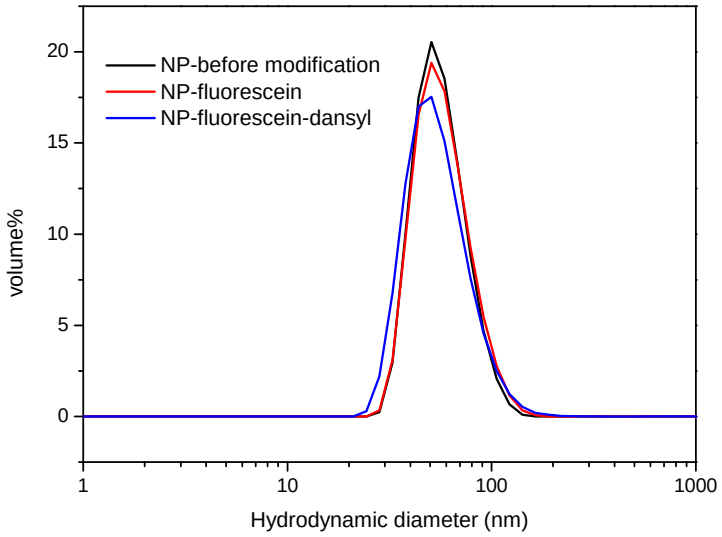
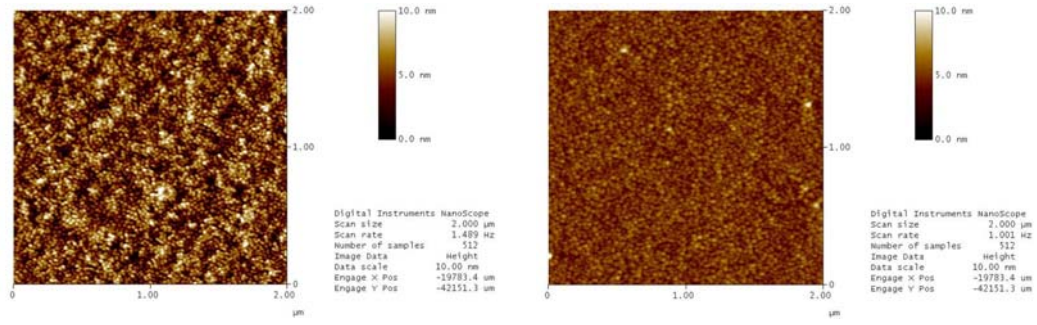


Fig.S9 DLS profiles of the original core-shell nanoparticles (NP-before modification), nanoparticles after one-pot aminolysis/Michael addition (NP-fluorescein) and nanoparticles after cascade aminolysis/Michael addition and click chemistry (NP-fluorescein-dansyl).

Characterisation of nanoparticles using AFM: AFM images were obtained using a Dimension 3000 (Digital Instruments) scanning force microscope in the tapping mode. AFM samples were prepared by dropping diluted (1:20) particle solutions onto silicon wafer, followed by drying under vacuum overnight.

A **B**



C

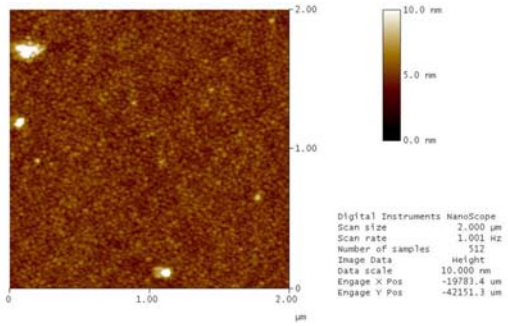
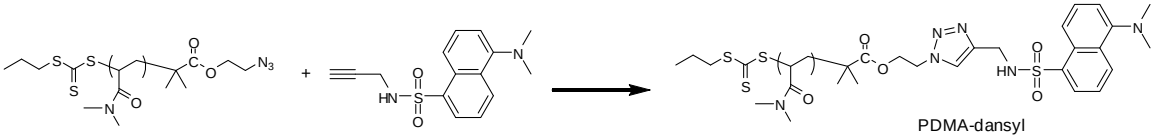


Fig.S10 AFM images of nanoparticles A) before modification, B) after modification with fluorescein and C) after modification with fluorescein and dansyl probe.



Synthesis of dansyl probe functionalised PDMA

A solution of PDMA (M_n = 6 900, M_w/M_n= 1.14) (0.2 g, 29.0 μmol) in 3 ml water was combined with 1.2 ml DMF solution of dansyl probe (25.0 mg, 87.0 μmol), followed by the addition of $CuSO_4 \bullet 5H_2O$ (1.5 mg, 5.8 μmol). After the resulting solution was degassed with argon for 1 h, 0.15 ml degassed sodium ascorbate solution (c=15 mg/ml, 2.3 mg, 11.6 μmol) was injected via a syringe. The resulting solution was stirred at room temperature overnight, purified by dialysis (MWCO 3 500) against frequently changed 20-40% ethanol solution for 2 days and then against frequently changed water for 2 days until UV-Vis measurements showed no absorption of dansyl probe. The polymer was isolated by freeze-drying at 60% yield. M_n = 7 700, M_w /M_n = 1.14. UV-Vis measurements of the modified polymer **PDMA-dansyl** using dansyl probe as standard in chloroform showed 49% functionalisation of the polymer with dansyl probe.

Quantification of fluorescein functionalisation in NP-fluorescein.

The content of fluorescein in **NP-fluorescein** was quantified as the amount of fluorescein in **NP-fluorescein** relative to the amount of trithiocarbonate groups in the original nanoparticles. First, a calibration curve was obtained using the absorption of fluorescein *o*-acrylate at 490 nm in pH = 7.0 buffer solution. Then, the absorption of 3 samples of **NP-fluorescein** at 490 nm at pH = 7.0 was used to calculate the fluorescein content of **NP-fluorescein** using the calibration curve.

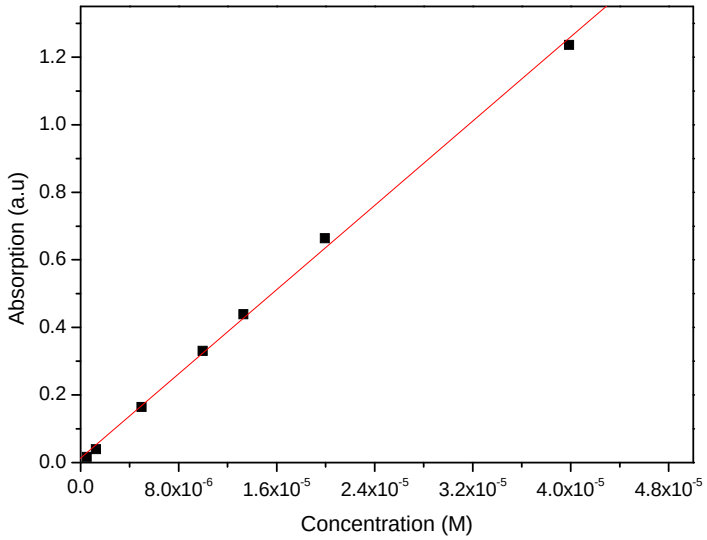


Fig.S11 Calibration curve of fluorescein *o*-acrylate in pH = 7.0 buffer solution using absorption at 490 nm.

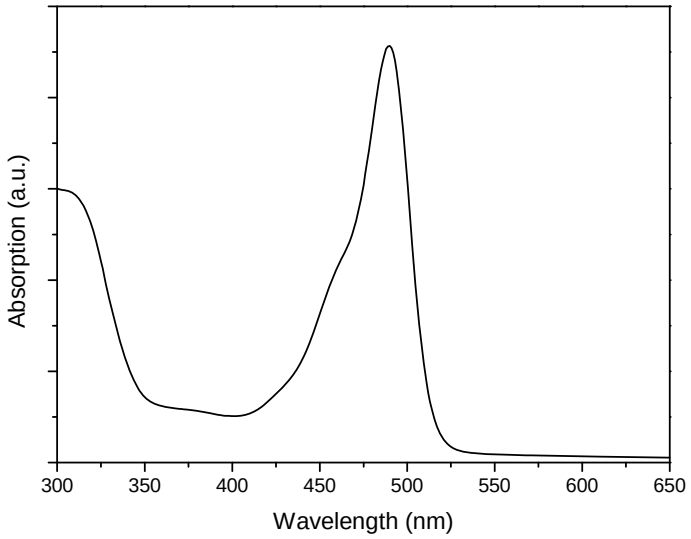


Fig.S12 UV-Vis absorption spectrum of NP-fluorescein in pH = 7.0 buffer solution.

Quantification of dansyl probe content in NP-fluorescein-dansyl.

First, a calibration curve was obtained using the absorption of dansyl probe at 370 nm in chloroform. Then, the functionalisation yield of dansyl probe in the polymer sample **PDMA-dansyl** was quantified as 49% using the calibration curve for **PDMA-dansyl** chloroform solutions. For quantification of dansyl probe in **NP-fluorescein-dansyl**, water soluble **PDMA-dansyl** was dissolved in pH = 7.0 buffer solution, which was used as the standard to quantify the content of dansyl probe in **NP-fluorescein-dansyl** by using their absorption at 370 nm in pH = 7.0 buffer solution. The measurements showed that the functionalisation yield of the nanoparticles with dansyl probe via click chemistry was essentially quantitative.

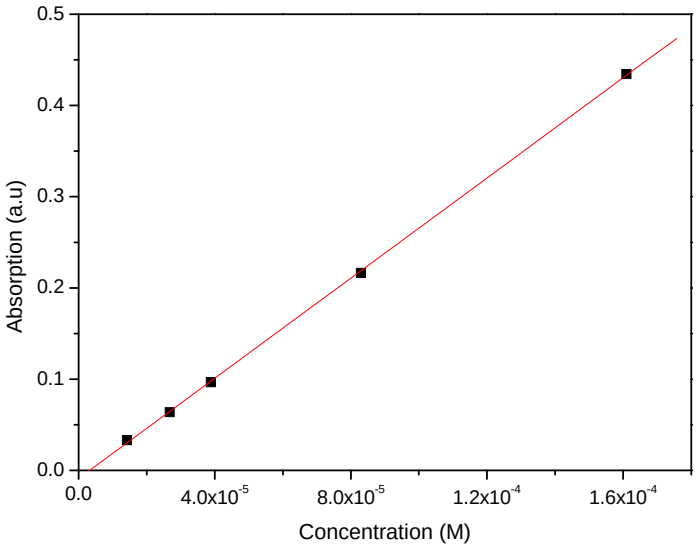


Fig.S13 Calibration curve of dansyl probe in chloroform using absorption at 370 nm.

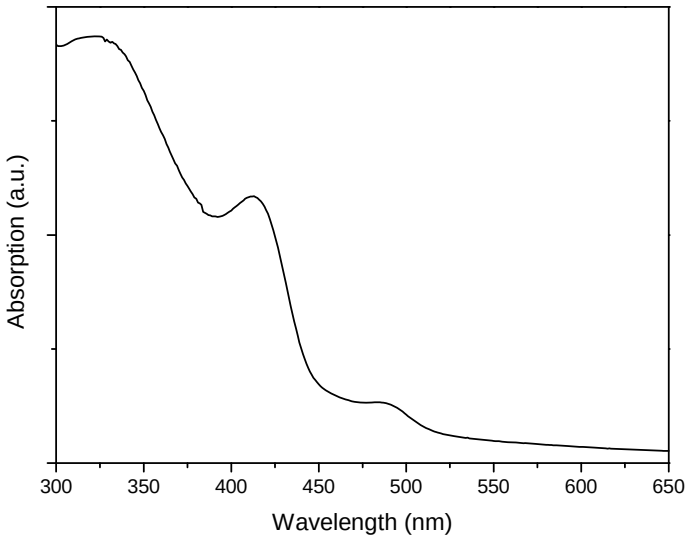


Fig.S14 UV-Vis absorption spectrum of NP-fluorescein-dansyl in pH = 7.0 buffer solution.

References

1. P. Kujawa, F. Segui, S. SHaban, C. Diab, Y. Okada, F. Tanaka, F. M. Winnik, *Macromolecules*, 2006, **39**, 341.
2. J. T. Lai, D. Filla, R. Shea, *Macromolecules*, 2002, **35**, 6754.
3. F. Bolletta, D. Fabbri, M. Lombardo, L. Prodi, C. Trombini, N. Zaccheroni, *Organometallics*, 1996, **15**, 2415.
4. Z. S. An, Q. H. Shi, W. Tang, C.-K. Tsung, C. J. Hawker, G. D. Stucky, *J. Am. Chem. Soc.*, 2007, **129**, 14493.