Supporting information for:

Functionalizable Amine-based Polymer Nanoparticles

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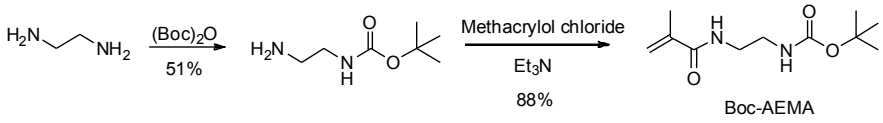
General:

Materials and Methods:

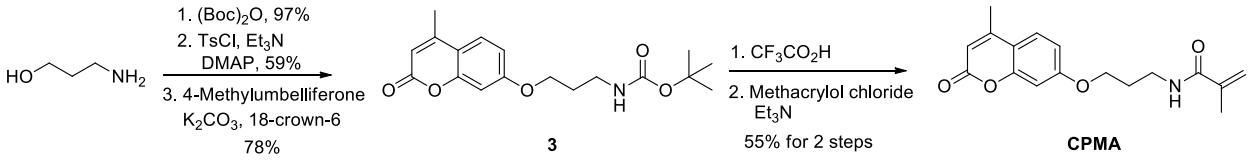
All chemicals and reagents were purchased from commercial sources and were used as received, unless otherwise mentioned. ^{1}H-NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer using the residual proton resonance of the solvent as the internal standard. ^{13}C-NMR spectra were recorded on a 400MHz Bruker NMR spectrometer using carbon signal of the deuterated solvent as the internal standard. ^{19}F-NMR spectra were collected on a 300 MHz Bruker NMR spectrometer. Molecular weights of the polymers were estimated by gel permeation chromatography (GPC) using PMMA standard with a refractive index detector. Dynamic light scattering (DLS) and zeta potential were determined by Nano-ZS (Malvern Instrument) Zetasizer. The fluorescence spectra were obtained from a JASCO FP-6500 spectrofluorimeter. UV-visible absorption spectra were collected using a Cary 100 spectrophotometer. FTIR spectra were recorded on a Perkin Elmer spectrometer. Contact angles of water were examined on a Ramé-Hart telescopic goniometer. Transmission electron microscopy (TEM) images were taken from JEOL 100CX at 100 KV. Atomic force microscopy (AFM) images were collected on a Digital Instruments Nanoscope III in tapping mode under ambient conditions by use of silicon cantilievers (spring constant 0.58 N/m).

Synthetic schemes for monomers:

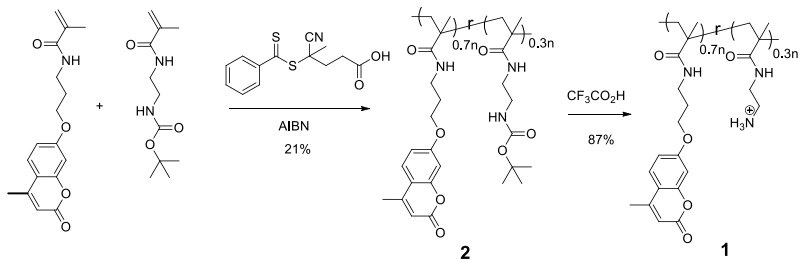
Synthesis of N-2-[(tert-butoxycarbonyl)amino] ethyl methacrylamide (Boc-AEMA)



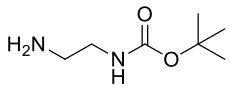
Synthesis of 4-Methylcoumarin-7-oxypropyl methacrylamide (CPMA)



Synthetic scheme for random copolymer 1:

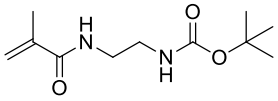


Synthesis of *N*-Boc-ethylenediamine:



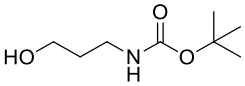
Di-*tert*-butyl dicarbonate (8.0 g, 36.7 mmol) was dissolved in chloroform (50 mL) and added dropwise to a solution of ethylenediamine (13.2g, 220 mmol) in chloroform (250 mL) at 0 °C. The mixture was allowed to warm to room temperature. After stirring for 12 hours, the reaction crude was filtered and washed with chloroform. The filtrates were collected and the solvent was evaporated. The crude was re-dissolved in ethyl acetate and washed with brine (3×100 mL) and water (100 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford *N*-Boc ethylenediamine (2.97 g, 51%) as a colorless oil. ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.95 (bs, 1H), 3.20 (q, 2H), 2.82 (t, 2H), 1.99 (s, 2H).[1]

Synthesis of N-2-[(tert-butoxycarbonyl)amino] ethyl methacrylamide (Boc-AEMA):



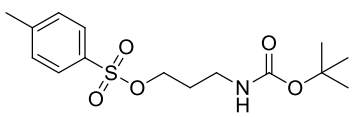
To a solution of N-Boc-ethylenediame (2.0 g, 12.5 mmol) in 20 mL of dry dichloromethane was added 1.5 g (15.0 mmol) of triethylamine and the mixture was cooled in an ice-bath. To this cold mixture, a solution of methacryloyl chloride (1.3 g, 12.5 mmol) in 10 mL dichloromethane was added dropwise with continuous stirring. After the addition, the reaction mixture was stirred at room temperature for 6 h. The stirring was stopped and the reaction mixture was washed with 3x30 mL distilled water and then with 30 mL of brine. The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated to get the crude product as a white solid. It was purified by column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. Yield: 2.52 g (88%). ^{1}H NMR (400MHz, $CDCl_3$) δ: 6.70 (bs, 1H), 5.75 (s, 1H), 5.33 (s, 1H), 4.92 (bs, 1H), 3.41 (q, 2H) 3.33 (q, 2H), 1.96 (s, 3H), 1.44 (s, 9H).[2]

Synthesis of Compound 3a:



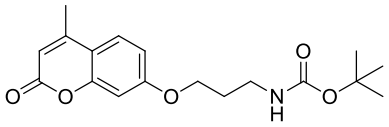
To a solution of 3-aminopropanol (2.0 g, 26.6 mmol) in chloroform (50 mL) was added di-*tert*-butyl dicarbonate (7.0 g, 31.9mmol) at 0 ^{o}C and stirred for 6h at room temperature. Chloroform was evaporated and the residue was re-dissolved in ethyl acetate and washed with saturated $NaHCO_3$ aqueous solution (100 mL) and brine (2 ×100 mL) The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to afford N-boc-3-aminopropanol (4.5 g, 97 % yield). ^{1}H NMR (400MHz, $CDCl_3$) δ: 3.66 (t, 2H), 3.29 (t, 2H), 1.66 (p, 2H), 1.44 (s, 9H).[3]

Synthesis of compound 3b:



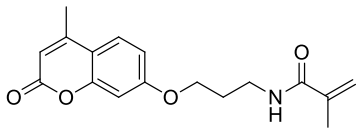
N-boc-3-aminopropanol (4.0g, 22.8 mmol) was dissolved in 100 mL of dry dichloromethane and 2.7g (27.4 mmol) of triethylamine was added to it. To this mixture, a solution of p-toluenesulfonyl chloride (5.2 g, 27.4 mmol) and 4-dimethylaminopyridine (catalytic amount) in 20 mL dry dicholoromethane was added. The reaction mixture was allowed to stir at room temperature overnight. Solvent was evaporated to get the crude product, which was purified by flash column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. Yield: 4.46 g (59 %). ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.80 (d, 2H), 7.36 (d, 2H), 4.10 (t, 2H), 3.16 (t, 2H), 2.45 (s, 3H), 1.84 (p, 2H), 1.42 (s, 9H).[4]

Synthesis of compound 3:



In a two-neck round bottom flask, compound **3b** (3.0 g, 9.1 mmol) was mixed with 4-methylumbelliferone (1.76 g, 10.0 mmol), K_2CO_3 (1.38 g, 10.0 mmol), and 18-crown-6 (0.48 g, 1.82 mmol) in acetone (300 mL) under argon atmosphere. The reaction mixture was refluxed for 12 hours. Then, the crude reaction mixture was filtered and washed with acetone. The filtrates were collected and the solvent was evaporated. The crude was then poured into water and extracted with ethyl acetate (3 x 100 mL). The organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. Yield: 2.36 g (78 % yield). ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.48 (d, 1H), 6.84 (dd, 1H), 6.80 (d, 1H), 6.13 (s, 1H), 4.73 (bs, 1H), 4.07 (t, 2H), 3.34 (q, 2H), 2.39 (s, 3H), 2.01 (p, 2H), 1.44(s, 9H). ^{13}C NMR (400MHz, $CDCl_3$) δ: 162.0, 161.5, 156.1, 155.4, 152.7, 125.7, 113.8, 112.7, 112.2, 101.58, 79.8, 66.3, 38.0, 29.7, 28.5, 18.8.

Synthesis of 4-Methylcoumarin-7-oxypropyl methacrylamide (CPMA):



To deprotect the N-boc amine functionality, compound **3** (2.36 g, 7.1 mmol) was dissolved in 10 mL of 1:1 v/v dichloromethane/trifluoroacetic acid mixture. After stirring at room temperature for 2 h, solvent mixture was removed by evaporation, and the oil residue was rinsed two times with diethyl ether (20 mL). The resultant precipitate was collected and dried in vacuo. To a solution of the dried precipitate in 50 mL of dry dichloromethane was added 2.15 g (21.3 mmol) of triethylamine and the mixture was cooled in an ice-bath. To this cold mixture, a solution of methacryloyl chloride (0.82 g, 7.8 mmol) in 10 mL dichloromethane was added drop-wise with continuous stirring. After the addition, the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was then washed with 3x30 mL distilled water and then with 30 mL of brine. The organic layer was collected, dried over anhydrous Na_2SO_4 and concentrated to get the crude product as a yellow solid. It was purified by column chromatography using silica gel as stationary phase and mixture of ethyl acetate/hexane as eluent. Yield: 1.18 g (55 %). ^{1}H NMR (400MHz, $CDCl_3$) δ: 7.50 (d, 1H), 6.84 (dd, 1H), 6.80 (d, 1H), 6.19 (bs, 1H), 6.14 (s, 1H), 5.71 (s, 1H), 5.34 (s, 1H), 4.12 (t, 2H), 3.55 (q, 2H), 2.39 (s, 3H), 2.10 (p, 2H), 1.97 (s, 3H); ^{13}C NMR (400MHz, $CDCl_3$) δ: 168.7, 161.8, 161.4, 155.3, 152.7, 140.0, 125.7, 119.8, 113.8, 112.4, 112.1, 101.6, 67.0, 37.5, 28.9, 18.8.

Synthesis of random copolymer 1:

A mixture of 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (7.8 mg, 0.028 mmol), Boc-AEMA (194 mg, 0.85 mmol), CPMA (600 mg, 1.99 mmol) and AIBN (0.92 mg, 0.0019 mmol) was dissolved in DMF (10 ml) and degassed by performing three freeze-pump-thaw cycles. The reaction mixture was sealed and then heated with a pre-heated oil bath at 75 °C for 12 h. The resultant mixture was precipitated in ethyl acetate (200 mL) to remove unreacted monomers. The precipitate was further dissolved in dichloromethane (5 mL) and re-precipitated in ethyl acetate (200 mL) to yield purified random copolymer as a yellow solid. Yield: 21%. ^{1}H NMR (400MHz, $CDCl_3$/MeOD) δ: 6.8-7.4, 6.5-6.8, 5.8-6.0, 3.8-4.1, 3.0-3.4, 2.1-2.4, 1.5-2.0, 1.2-1.4, 0.7-1.2. GPC (THF) M_n: 3000 Da. PDI: 1.3. The molar ratio between two blocks was determined by integrating the Boc group protons in Boc-AEMA and an aromatic proton in the coumarin and found to be 3:7 (Boc-AEMA:CPMA). To remove the Boc groups, the resulting random copolymer was dissolved in 10 mL of 1:1 v/v trifluoroacetic acid/dichloromethane mixture and stirred overnight at room temperature. Solvent mixture was then removed by evaporation, and the oil residue was rinsed three times with diethyl ether. The resultant precipitate was collected and dried overnight in vacuum to afford random copolymer **1**. Yield: 87%. ^{1}H NMR (400MHz, DMSO-d6) δ: 7.3-8.2, 6.7-7.1, 6.0-6.2, 3.9-4.2, 3.0-3.3, 2.7-2.9, 2.2-2.4, 1.5-2.1, 0.7-1.2.Complete disappearance of the methyl proton signal of the Boc group at 1.2-1.4 ppm confirmed that all the Boc groups have been removed.

Synthesis of azidoacetic acid

To a solution of sodium azide (2.3 g, 36 mmol) in water (50 mL) was added bromoacetic acid (1.0 g, 7.2 mmol) slowly. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with 1 M of HCl aqueous solution. The crude material was mixed with water (50 mL) and extracted with ethyl acetate (3 x 100 mL). The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford azidoacetic acid (0.39 g, 54% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ: 10.62 (bs, 1H), 3.98 (s, 2H).[5]

Synthesis of [2-(2-methoxyethoxy)ethoxy]acetic acid pentafluorophenyl ester (Peg178 PFP ester)

To a solution of [2-(2-methoxyethoxy)ethoxy]acetic acid (1 equivalent) and pentafluorophenol (1.2 equivalent) in dry dichloromethane was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 equivalent) and catalytic amount of 4-dimethylaminopyridine at 0 °C. The reaction mixture was stirred for 6 h at room temperature. The reaction mixture was washed with saturated $NaHCO_3$ aqueous solution and then with brine. The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the crude product. It was purified by column chromatography using silica gel as the stationary phase and mixture of ethyl acetate/hexane as eluent. Yield: 50%. ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.54 (s, 2H), 3.83 (m, 2H), 3.74 (m, 2H), 3.65 (m, 2H), 3.57 (m, 2H), 3.38 (s, 3H). ^{19}F NMR (300 MHz, CDCl3) δ: -152.5 (2F), -157.3 (1F), -161.9 (2F).

General procedure for the synthesis of N-hydroxysuccinimide (NHS) ester

To a solution of carboxylic acid (1 equivalent) and N-hydroxysuccinimide (1.2 equivalent) in dry dichloromethane was added 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.2 equivalent) at 0 °C and stirred for 12 hours at room temperature. The stirring was stopped and the reaction mixture was washed with saturated $NaHCO_3$ aqueous solution and then with brine. The organic solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the N-hydroxysuccinimide ester.

Methoxypolyethylene glycol 2,000 acetic acid NHS ester (PEG2000 NHS ester):

Synthesis of PEG2000 NHS ester was done in dry DMF and the crude reaction mixture was directly taken to next step without any purification.

Azidoacetic acid NHS ester:
Yield: 61%. ^{1}H NMR (400MHz, $CDCl_3$) δ: 4.24 (s, 2H), 2.88 (s, 4H).[5]

Lauric acid NHS ester:
Yield: 71%. ^{1}H NMR (400MHz, $CDCl_3$) δ: 2.84 (s, 4H), 2.60 (t, 2H), 1.74 (p, 2H), 1.40 (p, 2H), 1.20-1.35 (m, 14H), 0.88 (t, 3H).[6]

Capric acid NHS ester:
^{1}H NMR (400MHz, $CDCl_3$) δ: 2.84 (s, 4H), 2.60 (t, 2H), 1.74 (p, 2H), 1.17-1.48 (m, 12H), 0.88 (t, 3H).[7]

Nanoparticle preparation:
To a solution of random copolymer **1** (20 mg) dissolved in 200 uL of DMSO was added 19.8 mL of milliQ water. After sonicating for 2 h, the solution was filtered through a filter with a pore size of 0.22 µm. The polymer solution was adjusted to pH 3 in a scintillation vial and irradiated under a XX-15LW Bench Lamp (UVP) with UV light (365 nm) for 10 min. Crosslinking was monitored by the reduction of absorbance at 320 nm. In order to show that the synthesized nanoparticles are stable cross-linked networks, rather than the simple aggregation of the polymer, nanoparticles were redispersed in 1:9 H_2O/DMSO mixture in which DLS data shows a size of ~32 nm. The increase in size is likely due to the swelling of nanoparticles caused by DMSO. In contrast, DLS studies reveal no aggregates for the redispersion of non-crosslinked polymers in 1:9 H_2O/DMSO.

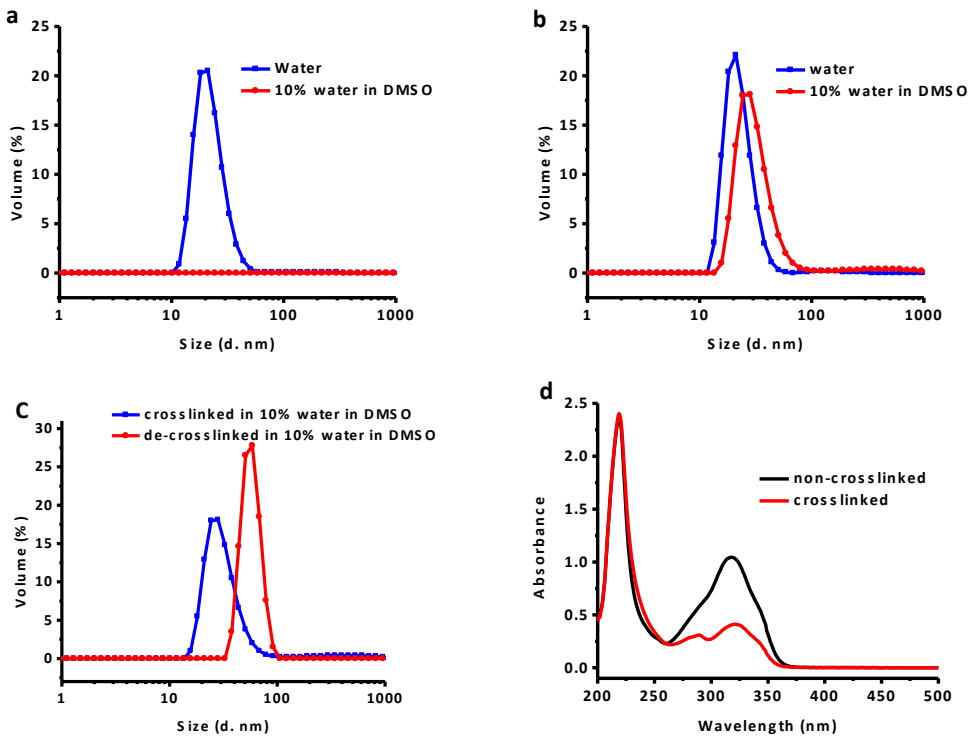


Figure S1. Size distributions of (a) non-crosslinked, (b) cross-linked, and (c) de-crosslinked random copolymer **1** by DLS. (d) Absorption spectra of non-crosslinked and cross-linked random copolymer **1**.

Encapsulation of guest molecules:
50 µL of 1mg/mL DiO/DiI (in acetone) was added to a vial, followed by evaporating the acetone with mild blow of air. To this was added 2 mL of nanoparticle solution (1 mg/mL) and sonicated at room temperature for 2 h. The resultant mixture was then passed through 0.22 µm filter to remove the non-encapsulated DIO/DiI, followed by stirring the solution at room temperature overnight to remove any residual acetone present in the solution. This stock solution was accordingly diluted with milliQ water (pH 3) to achieve required concentration of the nanoparticles.

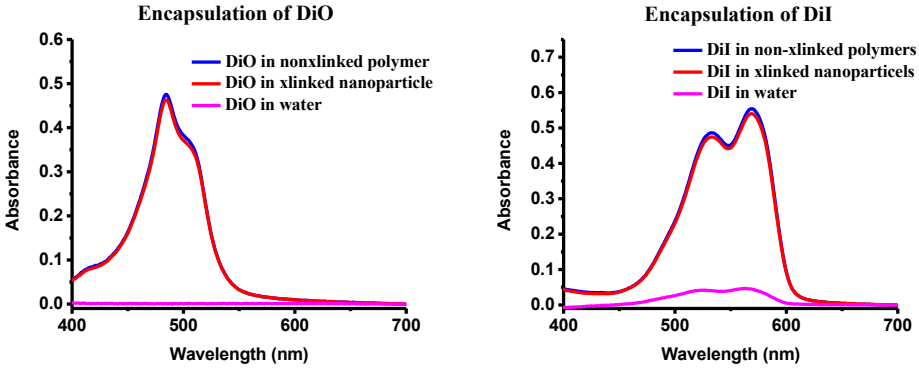


Figure S2. Absorption spectra of guest molecules in non-crosslinked and crosslinked random copolymer **1**.

General procedures for surface charge, contact angle, AFM and FTIR measurements:
To 1 mL of nanoparticle stock solution (1 mg/mL) at basic pH was added the functionalization agents (10 equivalents) dissolved in DMF. After stirring overnight at room temperature, the excess functional group reagents were removed by dialysis. For the functionalization with succinic anhydride, the reaction was done in 0.1 M NaCl solution to minimize the aggregation of opposite charge nanoparticles that could prevent the reaction from going to completion.

Surface charge measurements:
The reaction mixtures were first dialyzed in acetone to remove excess reagents and then were switched to aqueous medium. Solutions after dialysis were accordingly diluted with milliQ water to achieve a final concentration of 0.35mg/mL. All solutions were adjusted to pH 7.1 and then filtered through a 0.22 µm filter before performing surface charge measurements.

Contact angle and AFM measurements:
To prepare samples for contact angle and AFM measurements, stock solution of nanoparticles dissolved in water and dodecyl-functionalized nanoparticles dissolved in dichloromethane were dropped onto a silicon slides and dried at room temperature overnight.

Functionalization of nanoparticles for emission spectrum measurements:
Emission spectra were recorded on a JASCO (FP-6500) spectrofluorimeter using quartz cuvettes. To 100 µL of nanoparticle (1 equivalent) stock solution at basic pH, functional groups (3 equivalents) dissolved in DMSO (800 µL) were added and stirred overnight at room temperature. Fluorescamine (10 equivalents) dissolved in DMSO (100 µL) was then added and stirred for another 2 h at room temperature. All solutions were directly taken to the spectrofluorimeter for measurement without further purification. The emission spectra for fluorescamine-amine adduct were recorded by exciting at 390 nm, with both excitation and emission bandwidths set at 3 nm.

Quantifying the amounts of amine available for functionalization

Different aliquots of nanoparticle (200 µg/mL) were pipetted into a 96 well microplate in triplicates. Different volumes of water and DMSO were added to adjust the final water/DMSO (1:2, v:v) volume to 150 µL. The microplate was placed on a microplate shaker and 50 µl of 3.6 mM (1 mg/mL) fluorescamine dissolved in DMSO was added to each well. Following the addition of fluorescamine the plate was shaken for one minute and then allowed to stand at room temperature for 2 h. The fluorescence was then determined using a SpectraMax M5 plate reader with a 400 nm excitation filter and a 460 nm emission filter. The sensitivity setting was at 6 and the data collected from the top.

Different percentage of amines on the nanoparticles can be functionalized

100 µL aliquots of nanoparticle (200 µg/mL) were pipetted into a 96 well microplate in triplicates, followed by addition of different equivalents of capric acid NHS ester dissolved in DMSO to each well. Different volumes of water and DMSO were added accordingly to adjust the final water/DMSO (1:2, v:v) volume to 150 µL. The microplate was allowed to stay at room temperature for 6 hours during which it was shaken on a microplate shaker for the frequency of one minute per hour.

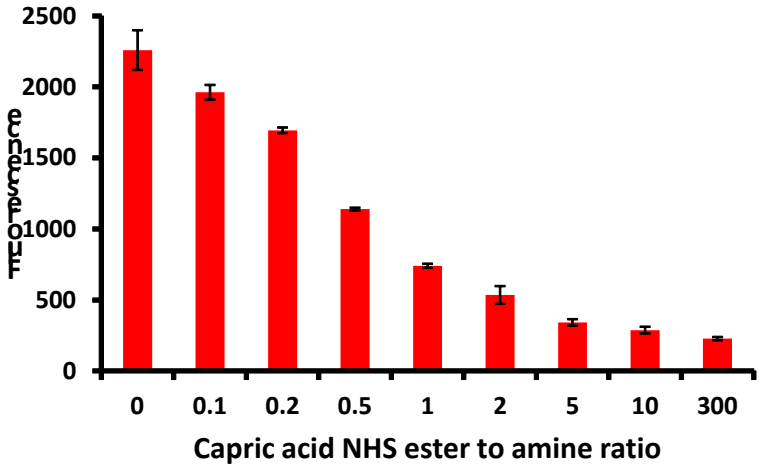


Figure S3. Nanoparticles reacted with different concentrations of capric acid NHS ester monitored by fluorescamine

Size control

2 mL polymer solutions (1 mg/mL) in scintillation vials were adjusted to the required pH using NaOH and HCl aqueous solution. After sonicating for 2 min, all solutions were irradiated under UV light (365nm) for 10 min to crosslink the polymers. The nanoparticle solutions were dialyzed in milliQ water to remove residual DMSO. All nanoparticle solutions were adjusted to pH 3 and then filtered through a 0.22 µm filter before performing dynamic light scattering measurements.

TEM image

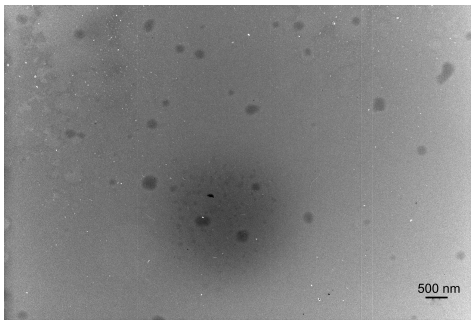


Figure S4. Size distribution of nanoparticles functionalized with capric acid NHS ester.

AFM images

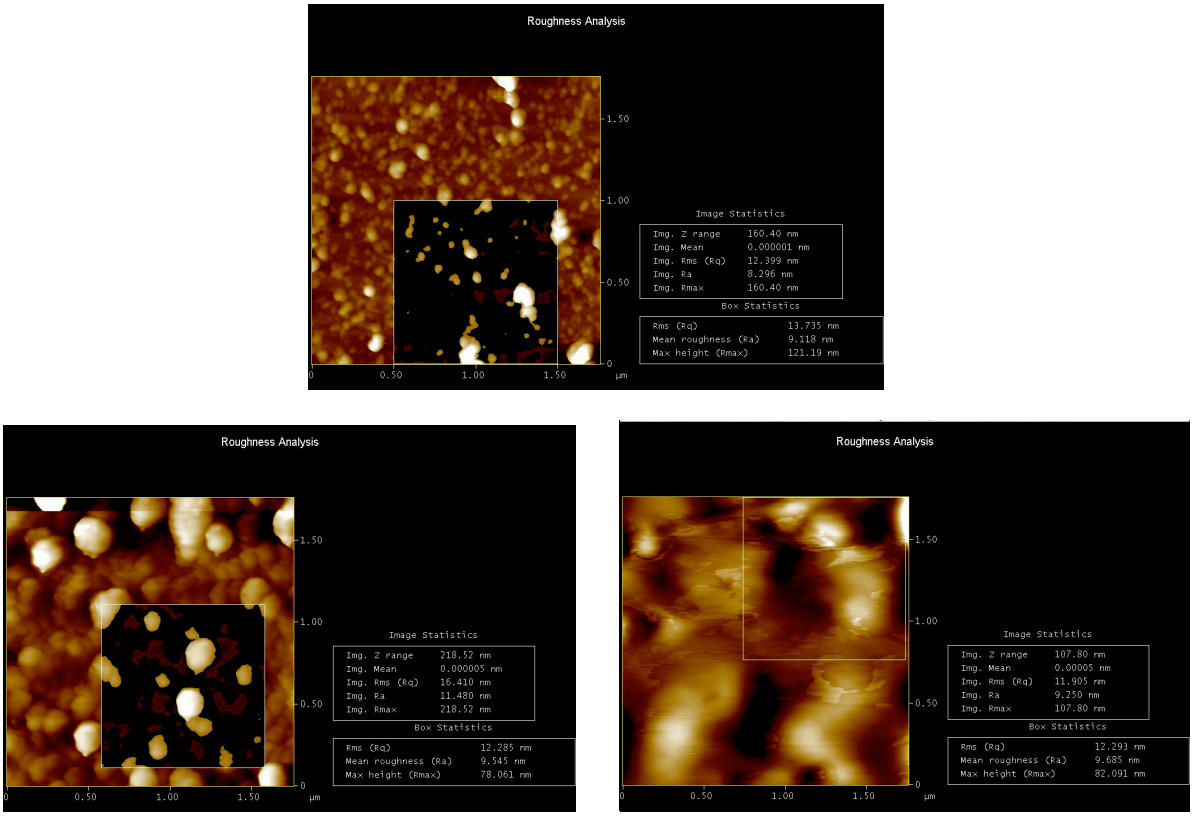


Figure S5. AFM images of unmodified nanoparticles (top) and nanoparticles modified by lauric acid NHS ester (bottom left) and dodecyl isocyanate (bottom right).

References:

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