Supporting information

for

Tuning the Catalytic Activity of L-Proline Functionalized Hydrophobic Nanogel Particles in Water

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Methods and materials

Materials. p-Toluenesulfonic acid monohydrate was purchased from Alfa Aesar. All other chemicals used were purchased from Sigma-Aldrich. Methyl methacrylate (MMA), ethyl methacrylate (EMA), ^{n}butyl methacrylate (*n*-BuMA), *tert*-butyl methacrylate (^{t}BuMA) and lauryl methacrylate (LMA) were filtered through a basic aluminium oxide column prior to use. 4-Nitrobenzaldehyde was filtered through a silica column prior to use. All other reagents were used without further purification.

Instrumentation. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker DPX 400 FT-NMR spectrometer using deuterated solvents. Chemical shifts are reported as δ in parts per million relative to $CHCl_3$ (7.26 ppm for ^{1}H) and $CDCl_3$ (77.2 ppm for ^{13}C) or CD_3OD (3.31 ppm for ^{1}H and 49 ppm for ^{13}C) as the internal standard. Dialysis tubing was purchased from Spectrum labs with molecular cut-off of 6-8 and 12-14 kDa. High performance liquid chromatography (HPLC) analysis was performed on a Varian 920-LC on an analytical column, Discovery C18 (100 mm×4.6 mm×5 µm) purchased from Sigma-Aldrich, UK and a chiral column, Chiralpak IA (150 mm×4.6 mm×5 µm) with guard cartridge (Chiralpak 5 µm) purchased from Chiral Technologies Europe. Hydrodynamic diameters (D_h) and size distributions of nanogels were determined by dynamic light scattering (DLS) on a Malvern Zetasized Nano ZS instrument operating at 25°C with a 4 mW He-Ne 633-nm laser module. Measurements were made at a detection angle of 173° (back scattering) and the data was analyzed using Malvern DTS 6.20. All determinations were made in triplicate (with 12 runs recorded for each measurement). Transmission electron microscopy (TEM) samples were prepared by drop deposition onto copper/carbon grids that had been treated with oxygen plasma to increase surface hydrophilicity and examined with a transmission electron microscope (JEOL TEM-2100), operating at 200 kV. Micrographs were collected at magnifications varying from 30 K to 100 K and calibrated digitally.

Experimental

*Synthesis of O-methylacryloyl-*trans*-4-hydroxy- L-proline hydrochloride*[1]

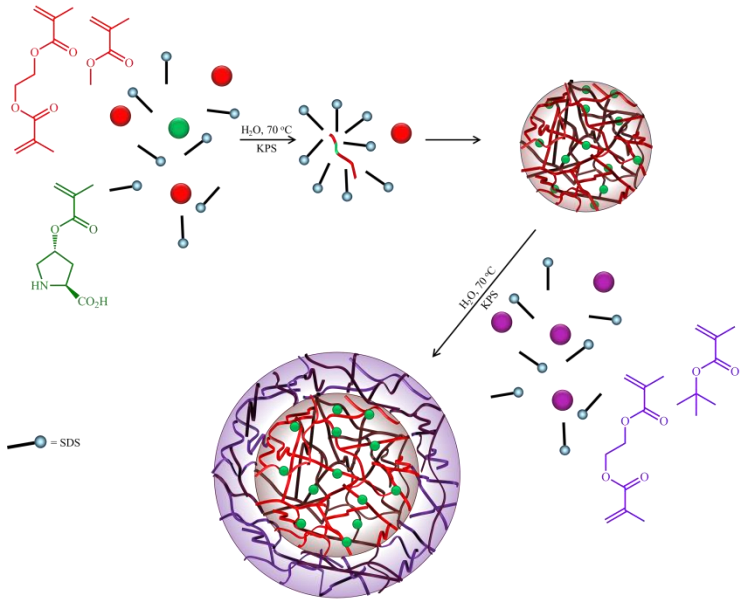
Hydroxy-L-proline (5.0 g, 38 mmol, 1.0 eq) was added in small portions to vigorously stirring trifluoroacetic acid (21.8 g, 191 mmol, 5.0 eq) at 0 °C. To the viscous solution, *p*-toluenesulfonic acid monohydrate (1.31 g, 7.6 mmol, 0.2 eq) was added and the resulting solution stirred for 5 minutes. Methacryloyl chloride (7.9 g, 76

mmol, 2.0 eq) was then added, resulting in a clear solution. The reaction was stirred for 3 hours at room temperature and subsequently cooled in an ice bath. Diethyl ether was then added dropwise and the resulting precipitate was filtered off using vacuum filtration, washed twice with additional cold diethyl ether and dried. The monomer was recrystallized using IPA/water (95/5), yielding white crystals (7.7 g, 91%). ^{1}H NMR (400 MHz, CD_3OD): δ = 1.86 (3H, t, C**H**$_3$, *J* = 1.3 Hz), 2.30-2.60 (2H, m, C**H**$_2$), 3.41 & 3.46 (2H, m, C**H**$_2$), 4.51 (1H, dd, C**H**, *J* = 10.5 & 7.8 Hz), 5.40 (1H, m, C**H**), 5.63 (1H, quin, C**H** vinyl, *J* = 1.5 Hz), 6.10 (1H, m, C**H** vinyl).

Preparation of functional nanogel particles

Hydrophobic functional nanogels: For a typical synthesis (0.5 wt% CLD, 2 wt% DoF), sodium dodecyl sulphate (SDS) (0.20 g) was dissolved in 100 mL nanopure water before adding the cross-linker, ethylene glycol dimethacrylate (EGDMA) (0.025 mL). L-Proline functionalized methacrylate (0.020 g) and the hydrophobic co-monomer methyl methacrylate (MMA) (1.0 mL) were then added under nitrogen flow with constant stirring. Finally, potassium persulfate (KPS) (0.005 g) was added and the reaction flask was immersed in a thermostatted oil bath set to 70 °C. After 12 hours continuous stirring at 800 rpm, polymerization termination was induced by cooling the reaction and allowing oxygen into the reaction vessel. Excess SDS was removed by dialysis against nanopure water.

Core-shell nanogels:[2] A 25 mL dispersion of the PMMA nanogels (50 wt% CLD) was heated to 70 °C and a degassed mixture of water (25 mL), SDS (0.063 g), ^{t}BuMA (0.125 g), EGDMA (0.125 g) and KPS (2.5 mg) were added under nitrogen using an automated syringe pump, at a rate of 25 mL/h. The polymerization was allowed to proceed for 12 hours at 70 °C before purification by dialysis against nanopure water to remove excess SDS.



Scheme S1. Schematic representation of the synthesis of the L-proline functionalized PMMA nanogels and the core-shell nanogels.

Aldol reactions: typical procedure

4-nitrobenzaldehyde (0.038 g, 0.25 mmol, 1 eq) was dissolved in cyclohexanone (0.104 mL, 1 mmol, 4 eq) and mixed with the functionalized nanogel (0.0025 mmol, 1 mol%). The reaction mixture was sonicated for 5 minutes and then stirred at room temperature for 24 hours. THF/acetone (1:5) was added at the end of the reaction to induce swelling of the pores, making it possible to extract the reagents from the core of the particles

into the organic phase. ^{1}H NMR spectroscopy was then used to determine the percentage conversion and product diastereomeric ratio (*anti/syn*). Crude product was filtered through a short silica column before the enantiomeric excess was determined using HPLC (ChiralPak IA, 90/10 hexane/isopropanol, 1.0 mL/min, major enantiomer t_R = 21.7 min, minor enantiomer t_R = 15.9 min).

The catalyst loading in usual reactions (i.e. 1 mol% catalyst = 0.498 mg catalyst) was determined using the concentration of catalyst in the nanogel solution, assuming 100% incorporation of the catalyst in the polymerization.

Table S1. Data for the catalysis experiments using nanogels with 2-15 wt% DoF.

DoF (wt%)	Cat. concentration (mg/mL)	Volume Required (mL)*
2	0.2	2.5
5	0.5	1.0
9	1.0	0.5
15	1.7	0.29

*Where less than 2.5 mL nanogel was required, this was then diluted with nanopure water to 2.5 mL, making the reagent concentration in each of the reactions identical.

For reactions where the same number of nanoreactors was used, the same volume of nanogel (2.5 mL) and amount of reagents (38 mg of 4-nitrobenzaldehyde and 0.104 mL of cyclohexanone) were used in each of the reactions. The catalyst loading in each reaction (mol% catalyst) was determined using the method described above and therefore, to give an example, in a reaction using nanogels with catalyst concentration of 1.7 mg/mL 4.25 mg of catalyst was used, which makes the catalyst loading 8.5 mol%.

Encapsulation experiments with Nile Red

Nile red (1.0 mg, 3.14×10^{-3} mmol) was dissolved in cyclohexanone (0.208 µL, 2.65 mmol) and split into 5 separate vials. To each vial a dispersion of nanogels in water (1.0 mL each) was added with varying DoFs and CLD. The nanogel dispersions were sonicated for 5 minutes, prior to stirring for 24 hours. Then, a small aliquot (0.05 mL) was taken and diluted with water (0.5 mL). Prior to analysis by fluorescence spectroscopy, each sample was filtered through a 0.45 µm syringe filter to remove any precipitated dye.

Determination of the monomer reactivity in water

Due to the different types of polymerization that MMA and ProMA undergo in water in the presence of a water soluble radical initiator (emulsion and precipitation respectfully), the conversion of the monomers was followed by ^{1}H NMR spectroscopy. A typical nanogel synthesis reaction was set up in the absence of the cross-linker (EGDMA). At predetermined time intervals 10 mL of the reaction mixture were withdrawn with a plastic syringe, dried and analyzed.

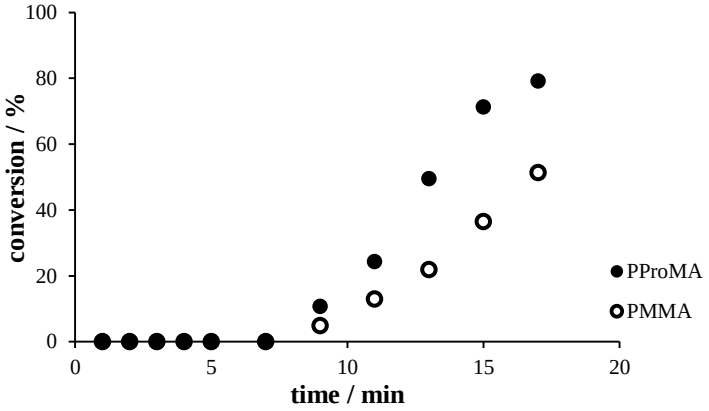


Fig S1. The copolymerization progress of L-proline functionalized methacrylate (15 wt%) and MMA was followed by ^{1}H NMR spectroscopy where the polymerization starts at the same time with functional L-proline monomer polymerizing slightly faster than MMA.

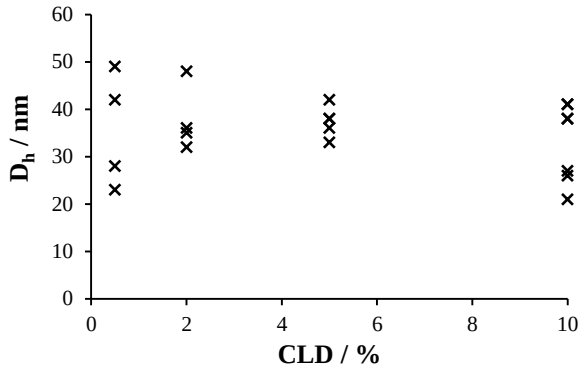


Fig S2. Graphical representation of particle size *vs* cross-linking density, determined by dynamic light scattering (DLS), for the range of PMMA nanogels with different CLDs (0.5-10 wt%) and DoF (2-15 wt%).

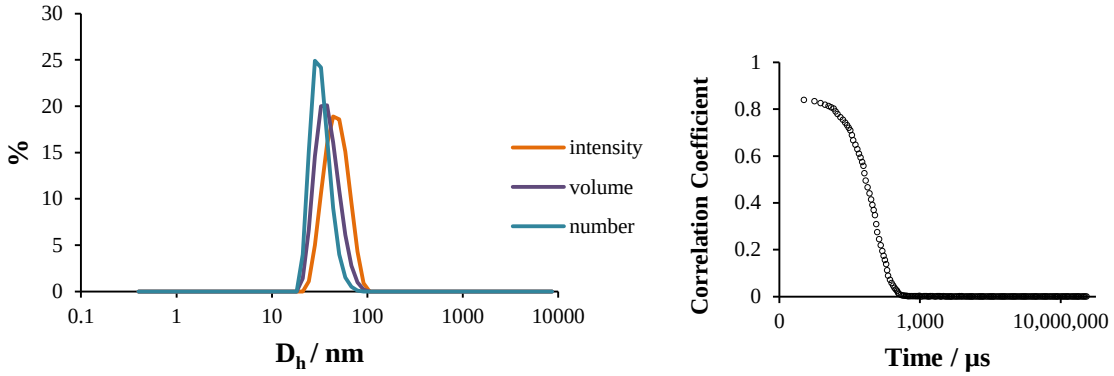


Fig S3. Representative DLS trace for functionalized nanogel particles (10 wt% CLD, 2 wt% DoF, 27 nm, PD 0.095) and the intensity correlation function.

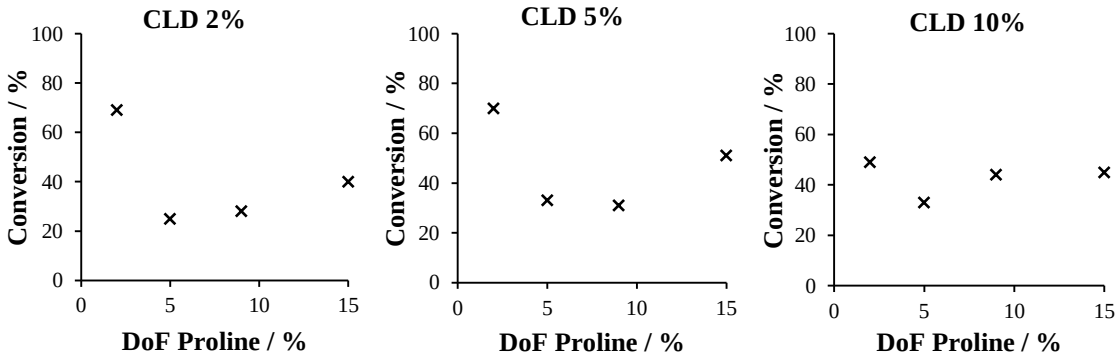


Fig S4. Effect of degree of catalyst functionalization (DoF) on conversion for reactions carried out with 1 mol% catalyst loading at room temperature after 24 hours for nanogels with varying CLD across the series shown.

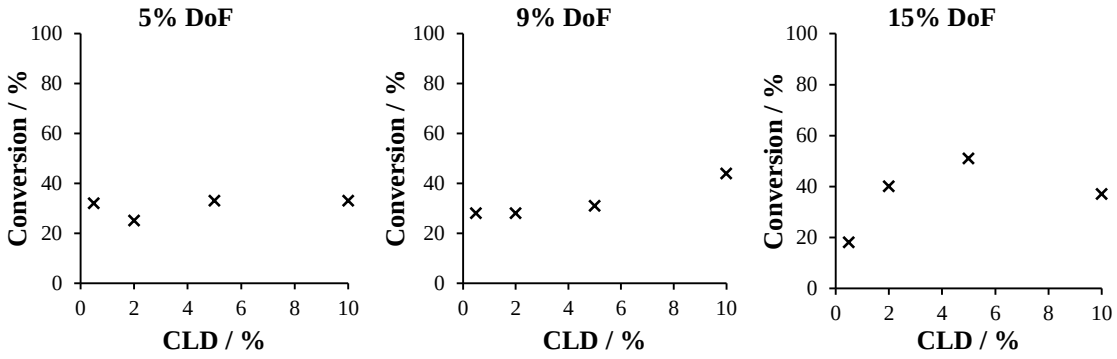


Fig S5. Effect of cross-linking density (CLD) on conversion for reactions carried out with 1 mol% catalyst loading at room temperature after 24 hours for nanogels with varying DoF across the series shown.

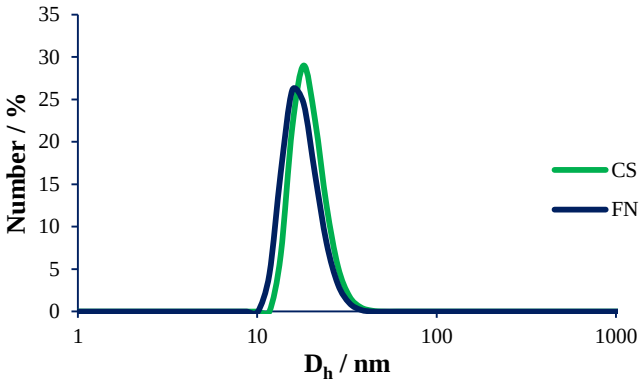


Fig S6. DLS trace of L-proline functionalized nanogel (FN, CLD 50 wt%, DoF 2 wt%) and its respective core-shell nanogel (CS, CLD 50 wt%, DoF 2 wt%, P^{t}BuMA shell CLD 50 wt%).

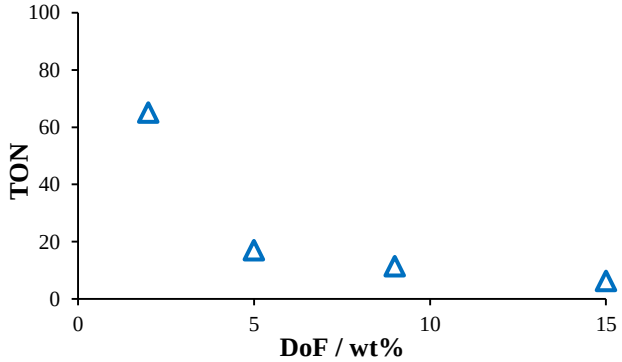


Fig S7. Aldol reaction carried out using the same number of PMMA nanoreactors (resulting in variable amounts of catalyst: 1-8.5 mol%), the catalyst efficiency is represented by TON.[3]

Control experiments

Control experiments using unfunctionalized PMMA nanogels (24 nm and PD 0.120) were carried out to ensure no background reactions were occurring simply due to the effective concentration of the two reagents within the hydrophobic core. In the absence of tethered catalyst (Entry 1), no reaction was observed and, even upon the addition of unsupported L-proline to the unfunctionalized PMMA nanogels (Entry 2), still no reaction was found, likely due to the high solubility of the catalyst in the surrounding water, essentially resulting in the segregation of catalyst and reagents. This emphasizes the importance of having the catalyst tethered within the particles bringing reaction components with different solubilities together within the same reaction sphere.

Table S2. Aldol reactions carried out in the presence of unfunctionalized PMMA nanogels with and without unsupported L-proline.

Entry	Nanogel	catalyst	mol%	conv.[a] / %	anti/syn ratio	ee / %
1	**unfunctionalized**	-	-	-	-	-
2	**unfunctionalized**	unsupported L-proline	10	-	-	-

[a] Determined by ^{1}H NMR spectroscopy, in $CDCl_3$

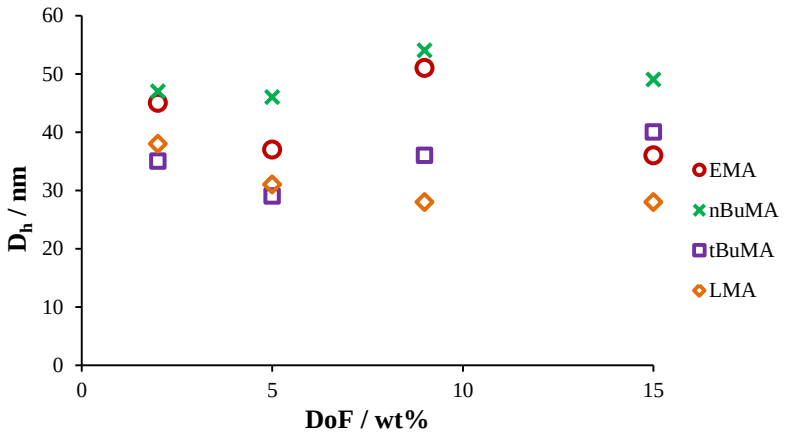


Fig S8. Size of L-proline functionalized nanogel particles (ethyl, ^{n}butyl, ^{t}butyl, lauryl methacrylate) with 0.5 wt% CLD and catalyst DoF in the range 2-15 wt%, as determined by DLS analysis.

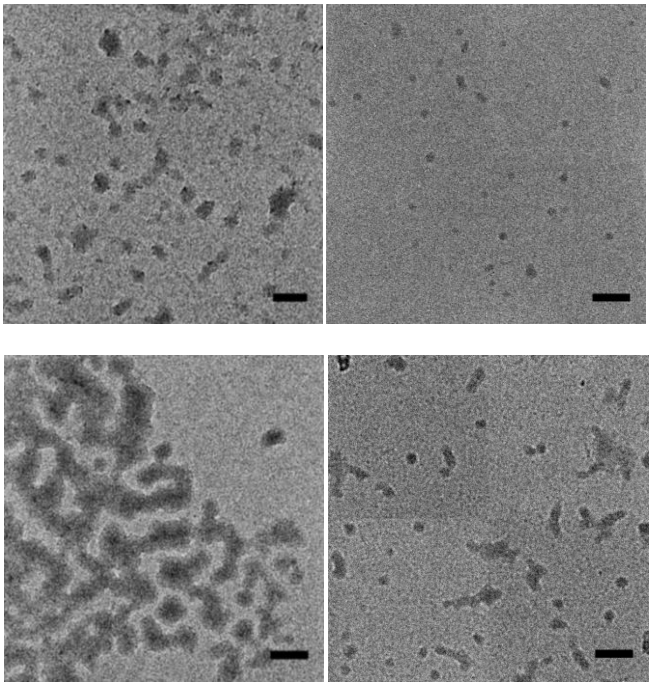


Fig S9. Unstained TEM micrographs of PEMA (D_{av} = 35 ± 5 nm), P^{t}BuMA (D_{av} = 20 ± 4 nm), P^{n}BuMA (D_{av} = 42 ± 6 nm) and PLMA (D_{av} = 22 ± 5 nm) nanogels, 0.5 wt% CLD, 2 wt% DoF, (scale bar = 100 nm).

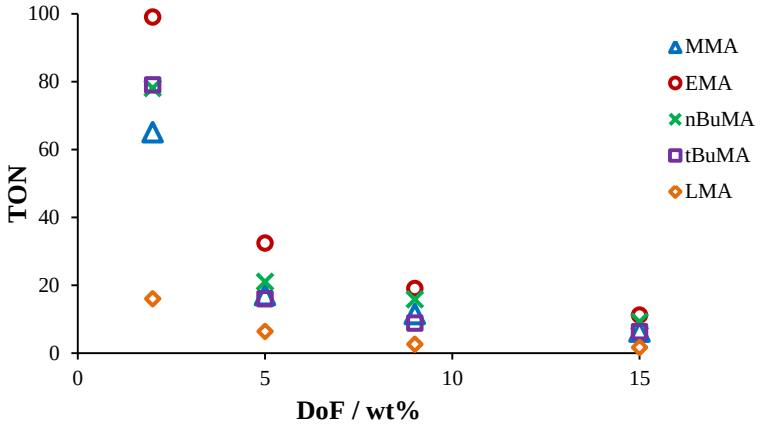


Fig S10. Catalytic efficiency (represented by TON) of functionalized nanogels with 0.5 wt% CLD and DoF ranging from 2-15 wt%; catalysis carried out at different catalyst loadings (1-8.5 mol%) but same number of nanoreactors.

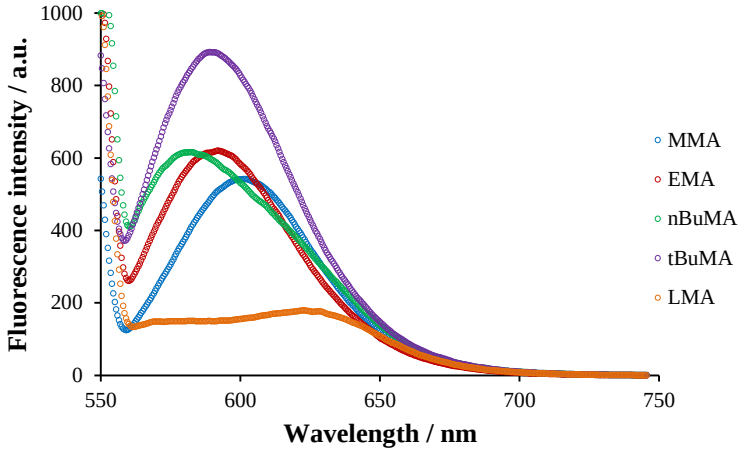


Fig S11. Emission spectrum (λ_{ex} = 550 nm) of nanogels (0.5 wt% CLD, 2 wt% DoF) with encapsulated hydrophobic dye, Nile Red (after filtration through 0.45 µm filter).

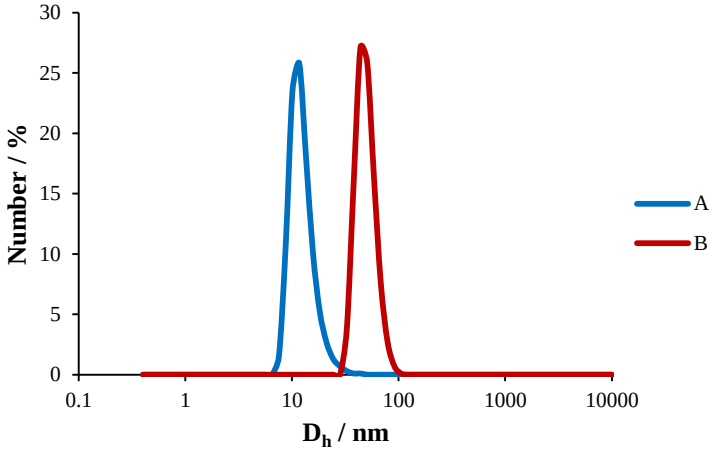


Fig S12. DLS trace of cross-linked PMMA nanogel (0.5 wt% CLD, 2 wt% DoF) A) stabilized by SDS in water, B) dried and re-dispersed in cyclohexanone, confirming the efficient uptake of cyclohexanone into the nanogel core resulting in swollen, larger particle size.

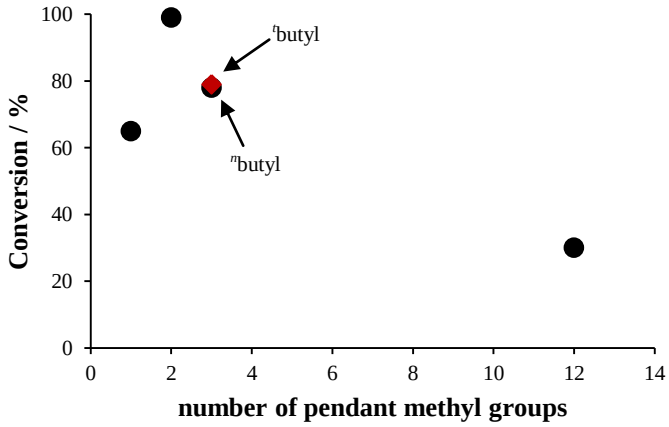


Fig S13. Reaction conversion after 24 hours determined by ^{1}H NMR spectroscopy, catalyzed by a range of nanogels presented in order of increasing number of pendant methyl groups on the non-functional co-monomer (i.e. methyl, ethyl, ^{n}butyl, ^{t}butyl (in red) and lauryl).

References

1. Optimised procedure based on: T. E. Kristensen, F. K. Hansen and T. Hansen, *Eur. J. Org. Chem.*, 2009, **2009**, 387.
2. K. E. Christodoulakis and M. Vamvakaki, *Langmuir*, 2009, **26**, 639.
3. Turnover number (TON) refers to the number of moles of substrates that one mole of catalyst can convert to product in a given time. In this case, it was determined for each nanogel system after 24 hours.