Controlled Fabrication of Polymer Microgels by Polymer-Analogous Gelation in Droplet Microfluidics

Supplemental Information: Experimental Details

Sebastian Seiffert and David A. Weitz
Harvard University
School of Engineering and Applied Sciences and Department of Physics
29 Oxford Street, Cambridge, MA 02138, U.S.A.
seiffert@seas.harvard.edu; weitz@seas.harvard.edu

Materials

N-(*N*'-Acryloyol-2-aminoethyl)-dimethylmaleimide (DMMIAAm, photocrosslinkable comonomer)

DMMI-functionalized acrylamide (DMMIAAm) was prepared according to Ref. 32: one amine group of 2-diaminoethane (Fluka) was blocked with Boc (Fluka), the other one was then reacted with dimethylmaleic anhydride (Aldrich), and, after removing the protecting group, the first amine group was reacted with acryloyl chloride (Merck). The following purification steps for the intermediate compounds and the final product were applied: BocNH–$(CH_2)_2$–NH_2 was purified by vacuum distillation at 58 °C/0.1 mbar, BocNH–$(CH_2)_2$–DMMI was recrystallized from water/ethanol (1:1), H_2N–$(CH_2)_2$–DMMI was intensely washed with methylene chloride, and AAm–$(CH_2)_2$–DMMI (DMMIAAm) was purified by recrystallization from hexane/ethyl acetate (1:1).

Poly-(*N*-isopropylacrylamide) (pNIPAAm)

All pNIPAAm homo- and copolymers discussed in this work were polymerized using the following free-radical polymerization scheme:

NIPAAm (ACROS Organics, recrystallized twice from hexane), sodium formate (Fluka), and, where appropriate, different types of comonomers were dissolved in water. The total monomer concentration was kept constant at 0.25 mol L^{-1} in all experiments, but the specific composition of the reaction mixture was varied as detailed below. After complete dissolution of all compounds, the mixture was purged with nitrogen for about 10 min at room temperature. The polymerization was initiated by adding 0.1 mol-% (relative to the total amount of monomers) of ammonium persulfate (APS, Aldrich) and 0.25 mol-% of *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TEMED, Fluka) in form of small amounts of appropriately concentrated aqueous solutions, and the mixture was stirred at room temperature under nitrogen atmosphere.

While the reaction was running, aliquots were withdrawn and mixed with similar amounts of methanol to check for a precipitation of pNIPAAm. This was done every 10 to 30 minutes. When a perceptible precipitation was noticed, the reaction was interrupted by adding an amount of methanol which equals the total volume of water in the reaction mixture. Conversions achieved by this procedure were about 10–30% in all cases. The precipitate was isolated by filtration, redissolved in water, and dialyzed against water for one week. Finally, the purified polymer was isolated by freeze drying.

Characterization of the product polymers was performed by NMR spectroscopy and size exclusion chromatography (SEC). Some analyses were also supplemented by UV spectroscopy and aqueous solution viscometry. ^{1}H NMR spectra were recorded on a Bruker Avance 400 digital FT-spectrometer at 400 MHz in either D_2O or DMSO-d_6. Chemical shifts reported in ppm are either referenced to residual H_2O using $\delta(H_2O)$ = 4.79 ppm[S0] or to internal trimethylsilane (TMS) using δ(TMS) = 0 ppm. SEC measurements were either performed utilizing ultrahydrogel columns filled with hydroxylated polymethacrylate-based gel together with a 0.02 wt.-% NaN_3 aqueous solution as eluent, or using four columns (100,000 Angström, 10,000 Angström, 1,000 Angström, and 500 Angström) and THF as eluent. The two systems were calibrated with pullulan or polystyrene standards. UV spectra were recorded on a Jasco V550 spectrometer. Aqueous solution viscometry at T = 20 °C was performed using an Ubbelohde viscometer with a capillary of type 0a (diameter 0.53 mm) and a series of four solutions with polymer concentrations of 0.5, 1, 1.5, and 2 g L^{-1}. Intrinsic viscosities, [η], were estimated from Huggins plots.

Molecular Weight Control of pNIPAAm

To check for the possibility to control the molecular weight of pNIPAAm, the polymerization procedure detailed above was applied to polymerize eight samples in the presence of different amounts of sodium formate as detailed in Table S1. Characterization of the product polymers was performed by aqueous size exclusion chromatography (SEC), as also compiled in Table S1.

Table S1. Impact of the presence of sodium formate on the molecular weight of pNIPAAm as obtained by free-radical polymerization in water. The monomer concentration during each reaction was 250 mmol L^{-1}.

Sample ID	***c*(sodium formate) in reaction mixture**	M_n	M_w	M_w/M_n
	mmol L^{-1}	g mol^{-1}	g mol^{-1}	
pNIPAAm-0	0	304,000	1,707,500	5.62
pNIPAAm-4	4	546,000	1,457,000	3.43
pNIPAAm-10	10	293,000	1,289,000	4.40
pNIPAAm-20	20	439,000	1,279,000	3.61
pNIPAAm-40	40	263,000	1,071,500	4.13
pNIPAAm-100	100	159,000	660,000	4.18
pNIPAAm-200	200	133,000	410,000	3.10
pNIPAAm-250	250	69,700	135,000	1.94

DMMI-Functionalized pNIPAAm

To prepare pNIPAAm precursors which are equipped with photocrosslinkable DMMI-sidegroups, the polymerization procedure detailed above was carried out in the presence of DMMIAAm. For the present work, two samples with different DMMI-contents and molecular weights were made. Table S2 lists the different compositions of the reaction mixture (Column 2–4). After polymerization, the molecular weight distributions of the products were estimated by size exclusion chromatography in THF using polystyrene calibration data. Additionally, the intrinsic viscosity, [η], was estimated by solution viscometry in water (T = 20 °C). The results of these analyses are compiled in Column 5–8 of Table S2. Furthermore, the DMMI-content of the two copolymers was estimated by ^{1}H NMR spectroscopy as detailed in Ref. 31:

^{1}H NMR (400 MHz, T = 100 °C, DMSO-d_6): δ = 0.80–2.25 (br, all backbone and methyl protons, 3.10–3.30 (br, xH, CONH–C<u>H</u>$_2$–CH$_2$–DMMI), 3.40–3.55 (br, xH, CONH–CH$_2$–C<u>H</u>$_2$–DMMI), 3.70–4.00 (br, 1H, C<u>H</u>(CH$_3$)$_2$), 6.50–7.00 (br, (1 + x/2)H, NH) ppm.

From the distinct integrals of the two CH_2 groups of the comonomer's dimethylene-subunit, x, the fraction of comonomer units in the copolymer was calculated. For this purpose, the integral of the methin proton within the *N*-isopropyl sidegroup of the NIPAAm main monomer (3.70–4.00 ppm) was defined to be 1.0. Then, x/2 represents the excess of comonomer units as compared to NIPAAm units.

To substantiate these analyses, UV spectroscopy data obtained in aqueous media were supplemented. From the characteristic absorbance of the DMMI-unit at λ = 229 nm, which was extracted from the polymer spectra by subtracting a spectrum of a reference sample containing pure pNIPAAm without any comonomers, the concentration of DMMI-moieties in the aqueous sample solutions was estimated. For the calculation with the Lambert–Beer equation, a molar absorption coefficient of DMMI-moieties of ε = 14,770 L·mol^{-1}·cm^{-1} (at λ = 229 nm)[28] was used. Knowing the amount of polymer in each sample solution then allows the relative DMMI-content in each polymer sample to be calculated. The results of these analyses are compiled in Column 9 and 10 of Table S2.

Table S2. Composition of the reaction mixtures for the synthesis of two NIPAAm–DMMIAAm copolymers, as well as characteristic data of the low-conversion products.

	Composition of reaction mixture			**Characterization of the resulting low-conversion products**					
Sample ID	**%$_{NIPAAm}$**	**%$_{DMMIAAm}$**	***c*(sodium formate)**	***M*$_n$**	***M*$_w$**	***M*$_w$/*M*$_n$**	**[η]**	**DMMI content (NMR)**	**DMMI content (UV)**
	mol-%	mol-%	mol L^{-1}	g mol^{-1}	g mol^{-1}		mL g^{-1}	mol-%	mol-%
p(NIPAAm–DMMI)-1	96.3	3.7	0	Not detectable			182	3.1	2.2
p(NIPAAm–DMMI)-2	98.8	1.2	0.2	100,000	200,000	2.0	98	0.79	0.74

FluorescentlyTagged pNIPAAm

Photocrosslinkable pNIPAAm precursors which are labeled with a red or green dye were prepared in a two step process. First, a terpolymer of NIPAAm, DMMIAAm, and an amine-functionalized comonomer, *N*-(3-aminopropyl)-methacrylamide (NAPMAAm, Polysciences), was prepared using the procedure detailed above. The reaction was carried out in the presence of 0.2 mol L^{-1} sodium formate, and the amount of DMMIAAm and NAPMAAm in the reaction mixture was 1.2 and 0.1 mol-% (rel. to the total amount of monomer), respectively. After isolating and characterizing the terpolymer by NMR and UV spectroscopy as well as SEC and aqueous solution viscometry, two fractions were labeled with either Alexa Fluor 488 succinimidylester (Molecular Probes) or carboxyrhodamine succinimidylester (Sigma Aldrich). For this purpose, 5 mg of either of these dyes were dissolved in two stocks of 2 mL anhydrous methanol. These solutions were mixed with two semidilute solutions of 450 mg of the p(NIPAAm–DMMI–NAPMAAm) terpolymer in 3 mL anhydrous methanol, respectively. The ratio of dye and free amine-moieties in each of these mixtures equals approximately two. The mixtures were shaken in the absence of light for 10 h and then quickly immersed in 2 × 100 mL of an aqueous 0.1 mmol L^{-1} Na_2CO_3/$NaHCO_3$ (1:100) buffer at pH 8.4, respectively. Initially, mixing these solutions led to a precipitation of the labeled polymers, but within 2 h all the material was re-dissolving. After letting the mixtures stand for additional 3 h at room temperature, the clear solutions were dialyzed against water for one week. Finally, the labeled products were isolated by freeze drying, and the tagged polymers were characterized by UV–vis spectroscopy. For this purpose, the absorbance of an aqueous solution of the labeled product was measured at the absorption maximum of the attached dye. Then, the molar concentration of the chromophores in the solution were calculated with the Lambert–Beer law using absorption coefficients of ε = 65,115 L mol^{-1} cm^{-1} (λ = 579 nm) for the carboxyrhodamine dye or ε = 73,000 L mol^{-1} cm^{-1} (λ = 494 nm) for Alexa Fluor 488. These analyses revealed a chromophore content of about 0.1 mol-% (rel. to the amount of monomer repeat units) in both the red- and the green-labeled polymer.

Sodium-thioxanthone-2,7-disulfonate (Triplet Sensitizer for Photocrosslinking)

Thioxanthone disulfonate (TXS) as introduced by Gupta *et al.*[S1] was chosen as water-soluble triplet sensitizer to crosslink the DMMI-containing precursor polymers. The properties of this compound have been studied by Kronfeld and Timpe,[S2] whose approach for synthesis and characterization was followed here.

Continuous Phases for Microfluidic Pre-Gel Emulsification

The continuous phase for all emulsification experiments discussed in this work was paraffin oil (Aldrich) containing 2 wt-% of a modified polyether–polysiloxane surfactant (ABIL EM 90, Evonik Industries, Germany). After vigorous stirring for several days, this mixture was filtered through a 450 nm PTFE filter before use.

Aqueous Phases for Microfluidic Pre-Gel Emulsification

All semidilute polymer solutions used for microfluidic emulsification were equilibrated for at least one week to ensure complete dissolution of the precursor material. Moreover, all solutions were filtered through a 5 μm PTFE filter before use.

Microfluidic Emulsification of Semidilute pNIPAAm Solutions and Formation of Microgels

Microfluidic Devices

Two types of microfluidic devices were employed to create pre-gel emulsions. One possibility is to use flow-focusing glass capillary devices. These consist of an internal cylindrical glass tube nested within a square glass capillary. In the region near the tip, an outer fluid (paraffin oil plus 2 wt.-% ABIL EM 90) focuses an inner fluid (aq. pNIPAAm solution, typical concentration 20–100 g L^{-1}, also containing TXS, typical concentration 0.5 mmol L^{-1}) through the tapered cylindrical collection tube. If the device is operating in a dripping state, this leads to the formation of monodisperse droplets that are carried downstream by the continuous phase. When double emulsions shall be formed, a second round capillary which is coaxially aligned to the collection capillary serves as injection tube for the inner phase (cf. Fig. 4D) in close proximity to the orifice of the collection tube. A more detailed description of the construction and the working principle of glass capillary microfluidic devices can be found elsewhere.[8,9,11]

A second possibility for microfluidic emulsification is to use polydimethylsiloxane (PDMS) microchannels. These can be made by soft lithography as detailed in Ref. 43. For the present project, a cross-junction geometry was used to obtain monodisperse droplets as shown in Fig. 4A. These droplets were carried downstream by the continuous phase.

In both cases, a hydrophobic surface needs to be created in the region where the drops form. This is necessary to avoid wetting of the dispersed aqueous phase on the device material. For the glass capillary devices, chemical treatment was applied by dipping the collection tube into octadecyltrimethoxysilane (Aldrich) before nesting it into the square capillary during device construction. To treat PDMS devices, Aquapel® (PPG, Pittsburgh, PA, U.S.A.), a commercial windshield treatment, was injected into the channels before using them. Both reagents were removed by thorough air drying before running the devices with the actual fluids.

Microfluidic Emulsification

All fluids were supplied using analytical glass syringes (Hamilton Gastight, 1–10 mL) and polyethylene tubing (Scientific Commodities). The flow rates were adjusted by syringe pumps (Harvard Apparatus, PHD 2000 series). To monitor the process of droplet formation, all devices were operated on a microscope (Leica DM IRBE) connected to a highspeed camera of type Phantom® (Vision Research).

For the experiments shown in Fig. 3, a glass capillary device with a tip diameter of 300 μm was used. Fluids were injected with flow rates of 1000 μL h^{-1} for the continuous phase, as well as 400, 200, 100, and 50 μL h^{-1} for the dispersed phase (from the uppermost to the lowermost micrograph in each stack of images in Fig. 3, respectively). For the fabrication of the monodisperse droplets shown in Fig. 4A, a PDMS microfluidic device with a cross-junction diameter of 50 μm was operated at flow rates of 160 mL h^{-1} for the continuous phase and 80 μL h^{-1} for the dispersed phase. To create double emulsions in the setup shown in Fig. 4D, a capillary device was employed. This device consisted of an injection tube with a tip diameter of 25 μm facing a collection tube with a tip diameter of 200 μm at a distance of 35 μm. The inner

phase (kerosene plus 2 wt.-% ABIL EM 90) was injected at 200 μL h^{-1}, the middle phase (aq. pNIPAAm solution plus 0.5 mmol L^{-1} TXS) was injected at 400 mL h^{-1}, and the outer phase (paraffin oil plus 2 wt.-% ABIL EM 90) was injected at 5000 μL h^{-1}. Varying the flow rate of the inner fluid to 150 μL h^{-1} and the flow rate of the middle fluid to 500 μL h^{-1} yielded double-core double emulsions, eventually leading to double-core microshells as shown in Fig. 4F.

Particle Curing and Workup

Once created, the pre-gel emulsions were exposed to UV light to solidify the aqueous drops. This was done as soon as possible to avoid droplet coalescence or splitting. When using PDMS devices, a geometry with a collection channel that gently widens and finally reaches a total diameter of 5 mm about 3 cm downstream (cf. Fig. 4A) was used. This area was exposed to a focused spot of strong UV light from a 100 W mercury arc lamp of type Omni Cure Exfo. Additional wavelength selection was performed by placing an optical filter into the beam path, the transmittance of which is limited to the range λ = 300–500 nm. The light intensity at the object level was 250 μW cm^{-2}. To avoid crosslinking of the precursor solutions before emulsifying them, all syringes and tubing were wrapped into aluminum foil. Moreover, the injection channel for the polymer phase was kept short, and as little PDMS as possible was used to construct the device to avoid extensive light scattering in the device material.

When using glass microcapillary devices, the freshly created emulsions were flown through a delay capillary (inner diameter 0.58 mm, length 14.5 cm) which was exposed to non-collimated UV light from a lamp of type Blak-Ray® (λ = 365 nm, intensity at the object level about 20 mW cm^{-2}). To protect the material from crosslinking before its emulsification, all tubing was wrapped into aluminium foil and all parts of the device which do not have to be monitored were masked.

All particle suspensions were collected in glass vials at the exit of the delay channel. To transfer the particles into plain aqueous environment, the supernatant oil phase was removed by depipeting, and five washing cycles with isopropanol were applied, followed by two washing cycles with 1,4-dioxane. Between these, particles were allowed to sediment. Depending on the size and density of the particles, this took several hours during the dioxane-washing phase. Finally, three washing steps with water were added. Note that during these, one has to be very careful, as adding water too roughly leads to the formation of air bubbles in the vial, which entails pronounced particle floating.

Crosslinking Efficiency and Gel Homogeneity

Oscillatory Shear Rheology

Oscillatory shear experiments were performed on a Bohlin CS plate–plate rheometer equipped with a UV-transparent bottom plate. All gel samples were prepared from pre-gel solutions placed between the plates. The setup was operated with a gap size of 0.5 mm and the diameter of the upper plate was 4 cm.

Photochemically crosslinked gels were prepared from 25, 50, and 100 g L^{-1} semidilute solutions of a precursor polymer with a DMMI-content of 3 mol-% by exposing the sample layers to UV light from the Blak-Ray® lamp mentioned above. By contrast, free-radically gelled samples with the same total concentration and comparable content of crosslinker (1.5 mol-% BIS, respectively) were prepared by reacting NIPAAm and BIS in a free-radical crosslinking copolymerization triggered by 10 mmol L^{-1} APS and 25 mmol L^{-1} TEMED.

In both cases, the progress of the reaction was monitored by continuous measurement of the rise of the elastic modulus, G', at T = 25 °C. After G' leveled off, its frequency dependence was probed by frequency sweeps in a range of ω = 0.01–100 Hz, using shear amplitudes which led to a total strain of 2%. From the plateau of $G'(\omega)$ at low frequencies, the zero shear modulus was obtained. From this value, the molar concentration of junctions, ν, was calculated using the theory of rubber elasticity as detailed in the main text.

Static Light Scattering

Nanoscale concentration fluctuations in the gels were estimated by static light scattering. For this purpose, 2 mL of semidilute precursor solutions (M_w = 200,000 g mol^{-1}, DMMI-content 0.75 mol-%) with concentrations of 25, 50, and 100 g L^{-1} were placed into cylindrical NMR tubes (Wilmad Labglass) with an inner diameter of 1 cm. Then, these samples were gelled at T = 25 °C by exposing them to UV light from a lamp of type Osram L 18W/73 (λ = 365 nm). The concentration of photosensitizer in the sample solutions was only 0.01 mmol L^{-1} to ensure an optical transmittance of more than 90% over a path length of 1 cm in the wavelength range employed. A concurrent spectroscopic check was kept on the progress of the photoreaction using a method detailed elsewhere.[28] For this purpose, small aliquots of each sample solution were placed in quartz glass cuvettes (Hellma) with a layer thickness of 0.1 mm, which were irradiated parallel to the light scattering cuvettes and used for UV–vis analyses to confirm completion of the photoreaction. For comparison, a set of free-radically crosslinked samples was prepared from solutions of NIPAAm and BIS (monomer concentrations 25, 50, and 100 g L^{-1}; ratios of NIPAAm and BIS as detailed in Tab. 2B) by adding 3.5 mmol L^{-1} APS and 16.7 mmol L^{-1} TEMED and polymerizing overnight at 25 °C.

Scattering intensities of all samples were estimated at 17 different angles between 40 and 150° using a SOFICA goniometer equipped with a HeNe laser (λ = 632.8 nm, T = 25 °C). To calculate the excess scattering intensities of the networks, $R_E(q)$, it is necessary to subtract the scattering intensity of an uncrosslinked, equally-concentrated semidilute solution, $R_{Sol}(q)$, from the intensity obtained from each crosslinked sample, $R_{Gel}(q)$. The $R_{Sol}(q)$ data were obtained by preparing reference samples in the absence of BIS in case of the free-radically gelling systems. For the photogelling samples, these data could be derived from the scattering data obtained from each sample cuvette prior to UV irradiation.

Analyzing the excess scattering intensities by the Debye–Bueche method yields the static correlation length and the root-mean-square fluctuation of the refractive index, which were transferred into mean square concentration fluctuations as described in Ref. 17. Fig. S1 shows plots of $R_E(q)^{-1/2}$ as a function of the scattering wave vector, q^2; these plots are well described by straight lines as predicted by the Debye–Bueche theory. The network parameters derived from the slopes and intercepts of the fitted lines (cf. Ref. 17 for details) are compiled in Table S3 for both the photogelling (A) and free-radically gelling (B) samples.

As detailed in the main text, it was intended to derive these results from samples exhibiting the same elastic modulus, respectively. To figure out which compositions had to be used to obtain such samples, separate estimates of *G*' were derived from the photogelling samples prior to the scattering experiments, using the same procedure as detailed above. Then, several free-radically gelled samples with the same total concentration but with differing molar fractions of BIS were examined until a set of compositions exhibiting the same *G*'-values as their photogelling counterparts was found. Eventually, these sample compositions were employed for the light scattering analyses detailed above.

Table S3. Network parameters obtained from the Debye–Bueche plots shown in Fig. S1. (A) Photogelling and (B) free-radically gelling samples. ξ denotes the static correlation length, whereas $\langle\delta\eta^2\rangle^{1/2}$ represents the root-mean-square fluctuation of the refractive index in the gels. From this quantity, the root-mean-square concentration fluctuation, $\langle\delta c^2\rangle^{1/2}$, can be derived using the refractive index increment of pNIPAAm.[17]

(A)

c_{total}	$c_{x\text{-}links,\ theoret.}$	ξ	$\langle\delta\eta^2\rangle^{1/2}$	$\langle\delta c^2\rangle^{1/2}$	$\langle\delta c^2\rangle^{1/2}$	G'	ν/ν_{theo}
g L^{-1}	mol-%	nm		g L^{-1}	%	Pa	%
25	0.375	14.6	$1.46 \cdot 10^{-6}$	7.42	29.0	70	3.5
50	0.375	10.7	$1.52 \cdot 10^{-6}$	7.38	14.8	925	22.7
100	0.375	11.6	$0.58 \cdot 10^{-6}$	4.60	6.1	3310	40.6

(B)

c_{total}	$c_{x\text{-}links,\ theoret.}$	ξ	$\langle\delta\eta^2\rangle^{1/2}$	$\langle\delta c^2\rangle^{1/2}$	$\langle\delta c^2\rangle^{1/2}$	G'	ν/ν_{theo}
g L^{-1}	mol-%	nm		g L^{-1}	%	Pa	%
25				No Gelation			
50	1.3	3.7	$30.1 \cdot 10^{-6}$	32.8	43.8	934	6.6
100	0.65	2.6	$89.4 \cdot 10^{-6}$	56.6	56.6	3300	23.1

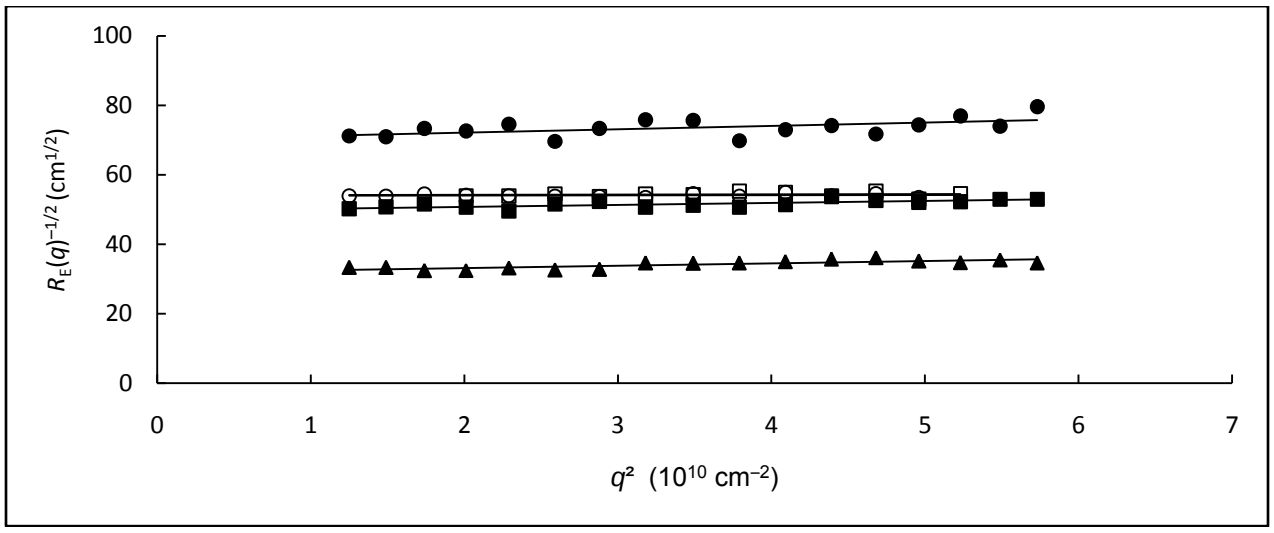

Figure S1. Debye–Bueche plots used to estimate the spatial inhomogeneity in pNIPAAm gel samples. Open symbols: free-radical crosslinking copolymerization. Solid symbols: polymer-analogous photogelation. Triangles: c = 25 g L^{-1}. Squares: c = 50 g L^{-1}. Circles: c = 100 g L^{-1}.

Optical Microscopy

To characterize the microgel micron-scale structures, particles were imaged with an optical microscope. To check for micron-scale inhomogeneities, three different samples were examined. First, microgels were made from a solution of 150 g L^{-1} NIPAAm, 1.5 mol-% BIS (rel. to the total amount of monomer), and 40 mmol L^{-1} APS. This solution was emulsified to monodisperse droplets of about 200 μm diameter using a flow focusing glass capillary device. The continuous phase in this experiment was a mixture of 90 vol.-% kerosene and 10 vol-% TEMED, also containing 80 g L^{-1} of a polyglycerol–polyricinoleate surfactant (PGPR90, Dow Corning). With this composition, droplet gelation occurred within a few seconds at room temperature.

Second, the same experiment was repeated, but this time using only 10 mmol L^{-1} APS in the aqueous and only 1 vol.-% TEMED in the continuous phase. The gelation of the droplets obtained from this experiment took several hours at room temperature.

Third, photogelled microgels were made from a pre gel-emulsion that consisted of paraffin oil plus 2 wt.-% ABIL EM 90 and aqueous droplets containing a 50 g L^{-1} solution of a pNIPAAm precursor material with 3 mol-% DMMI moieties. Crosslinking the polymers in this system took only a few seconds when using the 100 W mercury arc lamp of type Omni Cure Exfo mentioned above, along with a TXS concentration of 0.5 mmol L^{-1} in the aqueous phase.

After transferring them into plain water, all three types of microgels were imaged on a Leica TCS SP5 confocal laser scanning microscope operating in bright field mode to obtain the optical micrographs shown in Fig. 5.

Fabrication of Microgels with Defined Amounts of Fluorescent Tags

Microgels with well-defined amounts of fluorescent labels were made and characterized according to the following recipe:

First, four semidilute solutions of a crosslinkable pNIPAAm with a DMMI-content of 0.75 mol-% as well as additional amounts of the red- and green-tagged photocrosslinkable pNIPAAm precursors described above were prepared. The compositions of these solutions are compiled in Table S3. The solvent used was 0.5 mmol L^{-1} TXS in water in all cases. Then, these solutions were emulsified to 200 μm droplets using a glass capillary microfluidic device. Fluids were injected with flow rates of 1000 μL h^{-1} for the continuous phase (paraffin oil plus 2 wt.-% ABIL EM 90) as well as 150 μL h^{-1} for the dispersed phase. The droplets were gelled and worked up as detailed above. After suspending them in water, they were imaged on a Leica TCS SP5 confocal laser scanning microscope. Fluorescence excitation of the green (Alexa Fluor 488) and the red (carboxyrhodamine) dye was performed using either the 488 nm line of an Ar ion laser, operating at 0.36% of its maximum power (6 mW at the object level), or the 543 nm line of an HeNe laser, operating at 10% of its maximum power (0.3 mW at the object level). Fluorescence detection took place in two separate channels in the range of 501–535 nm (green channel, PMT gain 1250 V) and 558–638 nm (red channel, PMT gain 1250 V), respectively. All laser powers and photomultiplier currents mentioned above were adjusted such to obtain exactly the same average fluorescence intensity in the red and the green detection channel when imaging the microgels that contain solely 15 g L^{-1} red- or 15 g L^{-1} green-dyed material, respectively. To avoid crosstalk between the two channels, only one of the two fluorescence colors was exited and detected at a time.

The average fluorescence intensity in both channels was estimated for at least five particles of each kind using the analysis function of the Leica Software (LAS AF Version 2.1.0 build 4316). To ensure that the results are not impaired by photobleaching of the dyes during the UV crosslinking procedure, an independent check was kept on the photostability of the red- and green-tagged precursor polymers. This was done by extensive UV irradiation of 100 μm layers of the precursor solutions used for the present experimental series, accompanied by concurrent checks for potential changes in the dyes' absorbances by UV–vis spectroscopy. These experiments revealed no evidence for pronounced dye bleaching.

Table S3. Sample compositions for the production of red- and green-labeled microgels.

Sample ID	$c_{\text{untagged pNIPAAm}}$ / g L^{-1}	$c_{\text{green-tagged pNIPAAm}}$ / g L^{-1}	$c_{\text{red-tagged pNIPAAm}}$ / g L^{-1}
pNIPAAm-g15r00	35	15	00
pNIPAAm-g10r05	35	10	05
pNIPAAm-g05r10	35	05	10
pNIPAAm-g00r15	35	00	15

Additional References

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