Electronic Supplementary Material (ESI) for Materials Advances.

SUPPORTING INFORMATION

PNIPAAm microgels with defined network architecture as temperature sensors in optical traps

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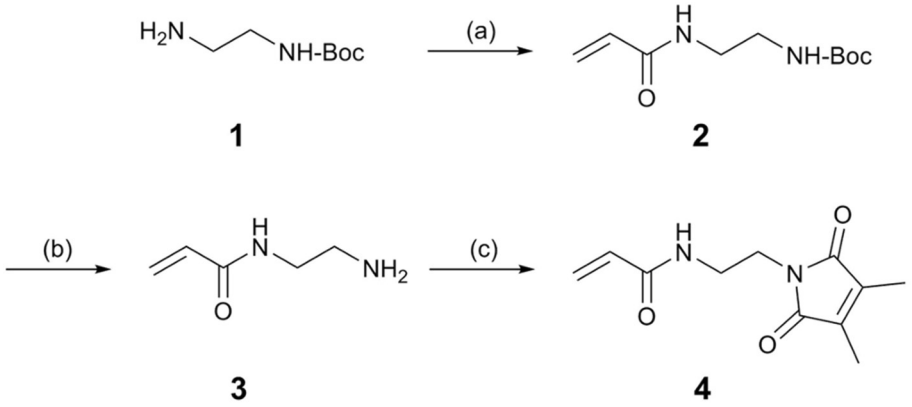
Synthesis of precursor PNIPAAm-co-DMMIAAm



Synthesis of *N*-tert-butyloxycarbonyl-*N*'-acryl-1,2-diaminoethane (**2**)

N-tert-butyloxycarbonyl-1,2-ethylenediamine (7.4 g, 46 mmol) was dissolved in dichloromethane (50 mL). After the addition of anhydrous sodium carbonate (4.9 g, 46 mmol), a solution of acryloyl chloride (4.2 g, 46 mmol) in dichloromethane (20 mL) was dropped slowly into this solution under continuous stirring and cooling in an ice/sodium chloride bath for 2 h. Subsequently, the mixture was stirred at room temperature overnight. On the next day, the mixture was filtered, the filtrate dried with magnesium sulfate, and the solvent was removed *in vacuo* to give **2** as a white solid.

Yield: 9.6 g (98%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.41 (br. s, 1H), 6.25 (d, *J* = 17.0 Hz, 1H), 6.09 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.63 (d, *J* = 10.4 Hz, 1H), 4.94 (br. s, 1H), 3.47 – 3.40 (m, 2H), 3.35 – 3.26 (m, 2H), 1.44 (s, 9H).

Synthesis of *N*-(2-aminoethyl)acrylamide (**3**) as TFA salt

2 (9.6 g, 45 mmol) was dissolved in dichloromethane (30 mL), trifluoroacetic acid (TFA, 100 ml) was added, and the mixture was stirred for 1 h at room temperature. The solvent was removed *in vacuo* to give **3** as TFA salt in quantitative yield.

^{1}H-NMR (500 MHz, DMSO): δ = 8.29 (br. s, 1H), 7.78 (br. s, 3H), 6.20 (dd, *J* = 17.1, 10.0 Hz, 1H), 6.11 (dd, *J* = 17.1, 2.2 Hz, 1H), 5.64 (dd, *J* = 10.0, 2.2 Hz, 1H), 3.39 – 3.33 (m, 2H), 2.94 – 2.85 (m, 2H).

Synthesis of *N*-[2-(3,4-dimethyl-2,5-dioxo-2,5-dihydro-pyrrol-1-yl)ethyl]acrylamide (DMMIAAm) (**4**)

3-TFA was dissolved in chloroform (60 mL). The pH of the solution was adjusted to >7 by addition of triethylamine (approx. 30 mL) to obtain the free base **3**. 2,3-Dimethylmaleic anhydride (4.7 g, 37 mmol) was dissolved in chloroform (120 mL) and added dropwise. The reaction mixture was stirred at 50 °C under argon for 23 h. After that, the mixture was filtered, washed with water (4 x 200 mL), and dried with magnesium sulfate. The solvent was removed *in vacuo* to give **4** as a whitish, yellowish solid.

Yield: 3.3 g (33%, related to 2,3-dimethylmaleic anhydride). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 6.28 – 6.20 (m, 1H), 6.07 (dd, *J* = 17.1, 10.3 Hz, 1H), 5.62 (dd, *J* = 10.4, 1.2 Hz, 1H), 3.73 – 3.68 (m, 2H), 3.57 – 3.45 (m, 2H), 1.96 (s, 6H).

Synthesis of poly {*N*-[2-(3,4-dimethyl-2,5-dioxo-2,5-dihydro-pyrrol-1-yl)ethyl)]acrylamide-co-*N*-isopropylacrylamide) (PNIPAAm-co-DMMIAAm)

N-isopropylacrylamide was recrystallized from hexane. Then, *N*-isopropylacrylamide (2.9 g, 25.9 mmol), **4** (303 mg, 1.4 mmol), and azobisisobutyronitrile (AIBN, 45 mg, 0.3 mmol) were dissolved in anhydrous *N*,*N*-dimethylformamide (DMF, 15 mL). After purging the solution with nitrogen for 10 min, the mixture was stirred under inert conditions for 24 h at 70 °C. The polymer was precipitated by dropping the reaction mixture into cold diethyl ether (400 mL). The precipitate was redissolved in a few mL of chloroform and precipitated into cold diethyl ether (400 mL) one more time to ensure complete removal of DMF and potential side products. Subsequently, the polymer was dried to a constant weight at 50 °C. Comparing integrals from ^{1}H-NMR spectroscopy ($CDCl_3$) belonging to the ethyl protons of the DMMI-acrylamide repeating units (3.61 ppm) and the *N*-isopropyl methyl protons of the NIPAAm repeating units (1.13 ppm) gave a DMMI-acrylamide fraction in the copolymer of 3.1 mol%.

Yield: 2.0 g (61%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 3.99 (br, *x*H, N-isopropyl CH protons), 3.61 (br, 4H, DMMI CH_2 protons), 2.34 – 1.25 (br, *x*H, all backbone protons + DMMI CH_3 protons), 1.13 (s, *x*H, N-isopropyl CH_3 protons). SEC [Poly(2-vinylpyridine) standard]: M_n = 25.000 g mol^{-1}, M_w = 113.000 g mol^{-1}, M_w/ M_n = 4.52.

Synthesis of photosensitizer TXS

Synthesis of sodium thioxanthone-2,7-disulfonate (TXS)

TXS was synthesized based on a protocol from Kronfeld and Timpe.[1] Briefly, thioxanthen-9-one (5 g, 24 mmol) was dissolved in 100 mL fuming sulfuric acid with 20% SO_3 content (H_2SO_4 fum.). The solution was stirred at 120 °C overnight. After cooling to room temperature, the solution was poured over 350 g of ice. The excess of H_2SO_4 fum. was removed *via* neutralization with calcium carbonate ($CaCO_3$) as gypsum. The dispersion was filtered, and barium chloride dihydrate ($BaCl_2 \cdot 2\ H_2O$, 5 g, 20 mmol) was

dissolved in 10 mL of DI water and added to the clear filtrate. TXS barium salt directly precipitated from the solution, was filtered off, and recrystallized three times from DI water. Subsequently, the barium salt was dissolved in a few mL of hot DI water, and a solution of sodium carbonate (Na_2CO_3), dyed with phenolphthalein, was added until the solution showed a slightly red color. The resulting precipitate was filtered off and the filtrate concentrated *in vacuo*. 2 N sulphuric acid was added until the red color vanished, and the precipitate was filtered off. Then, the solvent was completely removed *in vacuo*. The sodium salt was ground, washed several times with acetone, and dried to constant weight at 80 °C. The product was obtained as a yellow solid.

Yield: 0.9 g (10%, related to $BaCl_2 \cdot 2\ H_2O$). ^{1}H-NMR (500 MHz, D_2O): δ = 8.82 (d, *J* = 1.9 Hz, 2H), 8.04 (dd, *J* = 8.5, 2.1 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H).

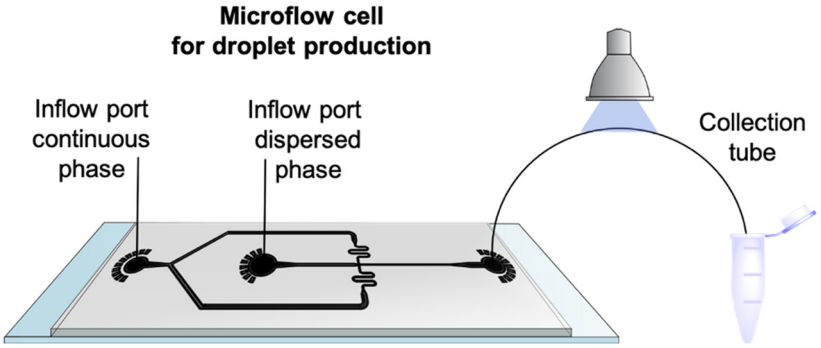


***Figure SI-2**. Scheme of the experimental setup for PAG microgel fabrication. AutoCAD sketch of a microflow cell for droplet generation connected via tubing to an Eppendorf collection vial, in which the formed emulsion is collected. The emulsion is irradiated in the outflow tubing with UV light directly from the light guide of a UV source. Details regarding the UV source can be found in the Materials and methods section.*

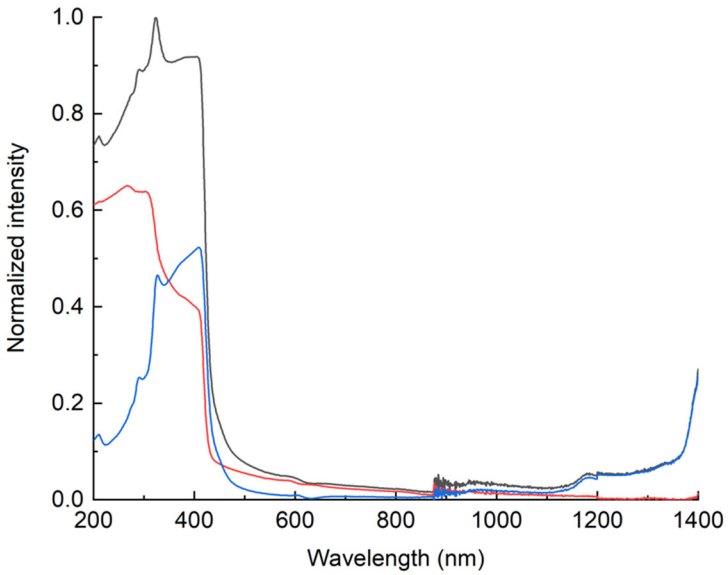


***Figure SI-2**. UV-vis-IR-spectra of extinction (black), scattering (red), and absorption (blue) of a PNIPAAm PAG hydrogel in D_2O calculated by measuring the complete scattering response and the scattering response without the zero-order*

transmission. D_2O was used as a solvent and reference to avoid distortion of the weak scattering signal in the IR by the scattering of H_2O stretching vibrations.

Calculation of the longitudinal modulus

The longitudinal modulus for different temperatures was calculated *via* the following formula:

$$M = \frac{{\nu_B}^2 \lambda^2 \rho}{4n^2}$$

Here, ν_B is the measured Brillouin shift, and λ is the incident laser wavelength. Refractive indices n for each temperature were extracted from the quantitative phase images. The corresponding density ρ was calculated by applying a binary mixture model for the microgels at room temperature.[2] This model establishes a linear relationship of density and refractive index employing a refractive index increment α. The value of α was set to 0.18, which is a good approximation for celluar content,[3] as well as for polyacrylamide gels.[4]

$$\rho = \frac{n - n_{water}}{\alpha} + \rho_{water}$$

Density values for higher temperatures, where preconditions for the mixture model are not provided, were calculated based on the value at room temperature assuming a constant polymer mass for all investigated temperatures. This assumption is justified by the fact that the PNIPAm beads recover their volume after a thermally induced shrinkage completely. This indicates that only water leaves the beads during their collapse.

1 K.-P. Kronfeld and H.-J. Timpe, *Journal für Praktische Chemie*, 1988, **330**, 571–584.
2 R. Schlüßler, S. Möllmert, S. Abuhattum, G. Cojoc, P. Müller, K. Kim, C. Möckel, C. Zimmermann, J. Czarske and J. Guck, *Biophysical Journal*, 2018, **115**, 911–923.
3 R. Barer, K. F. A. Ross and S. Tkaczyk, *Nature*, 1953, **171**, 720–724.
4 J. Stejskal and J. Horská, *Die Makromolekulare Chemie*, 1982, **183**, 2527–2535.