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Tetra-sensitive graft copolymer gels with high volume changes†

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For the preparation of multi-responsive graft copolymer gels for hydrogel-based microsystem technologies, a poly(4-vinylbenzoic acid) macromonomer was prepared in a three-step synthesis. At first, a chain transfer agent was functionalized with an azide group, followed by a reversible addition fragmentation chain transfer polymerization to give an azide bearing poly(4-vinylbenzoic acid) macromonomer. Subsequently, an acrylamide functionalized macromonomer was prepared by Cu-catalyzed alkyne and azide 1,3-dipolar cycloaddition. With this acrylamide functionalized poly(4-vinylbenzoic acid) macromonomer, various graft copolymer gels composed of a temperature-sensitive poly(*N*-isopropylacrylamide) backbone and pH-sensitive poly(4-vinylbenzoic acid) graft chains were prepared by radical polymerization. Moreover, the swelling behavior of the hydrogels was investigated with respect to pH, temperature, solvent and salt. It was shown that the graft copolymer gels respond to these stimuli independently with a sharp transition and a high volume change. Additionally, the swelling behavior was reversible over various cycles. This behavior is fundamental for applications of smart gels in microfluidic platforms.

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Introduction

Hydrogels are three-dimensional, cross-linked networks of hydrophilic polymers able to absorb and retain large amounts of water. It is possible to prepare "smart" hydrogels by using responsive monomers, which change their volume in response to alterations of certain environmental stimuli. These stimuli can be classified into three categories:[1] physical (*e.g.* temperature, ionic strength, electric fields), chemical (*e.g.* pH, specific ions) or biomedical (*e.g.* enzyme substrates, affinity ligands). Due to the ability to control and tailor the properties of these materials, hydrogels are very attractive for a wide range of applications such as in biomedicine (drug delivery system),[2–5] catalysis,[6–8] membranes,[9–11] cell culture support,[12,13] and as actuator/sensor materials in microfluidics.[14–16]

Microfluidics is a relatively new field of interest with high potential in multiplexing, automation and high-throughput screening.[17,18] Here, the incorporation of stimuli-responsive hydrogels as chemomechanical valve is highly promising because hydrogels combine sensor and actuator abilities in one element. With these key characteristics, no additional power source is needed and complexity of microfluidics platforms can be significantly reduced.[16,19,20] In addition to that, it would be beneficial and ideal if the hydrogel could respond to multiple stimuli. Optimally, these stimuli should be addressable independently with a sharp transition and a high volume change. In this regard, a combination of the stimuli temperature and pH is preferred since the regulation function of the gel can be carried out by an external control unit (temperature) as well as by a chemical information in the substrate flow (pH change).

It has been shown that temperature-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogels are capable for flow control.[14,21] Control over the hydrogel function is caused by the coil-to-globule transition of PNIPAAm above the lower critical solution temperature (LCST) around 32 °C in water accompanied by a macroscopic phase separation.[22–25] Consequently, PNIPAAm hydrogels exhibit a similar transition near 32 °C associated with high shrinking in volume. Note that the critical temperature for hydrogels is called volume phase transition temperature (VPTT). The most common approach to achieve a multi-responsive material is the copolymerization. It is well known that small amounts of hydrophilic comonomers increase the LCST of PNIPAAm, while hydrophobic comonomers decrease the latter value.[24,26] However, a limitation of PNIPAAm hydrogel is its poor tolerance of comonomers.[23,27] Above a critical comonomer content (5–10%), PNIPAAm hydrogels exhibited a declining temperature sensitivity caused by the increasingly interruption of the PNIPAAm segments by comonomer units. In order to overcome this drawback different approaches have been investigated such as semi-

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interpenetrating networks,[28] interpenetrating networks,[29–31] or graft copolymer gels.[27,32–37] Graft copolymer gels by free radical polymerization were first reported in 1995 by Okano *et al.*[36] It has been shown that *net*-PNIPAAm-*g*-PNIPAAm hydrogels exhibit enhanced deswelling kinetics compared to *net*-PNIPAAm hydrogels. Since then, several studies have confirmed the improved swelling kinetics of grafted hydrogels.[27,37] This ability is highly demanded from the application point of view because it accelerates the potential response time of the hydrogel as a chemomechanical micro-valve. Another strategy for preparing graft copolymer gels is to use controlled/living radical polymerization (CRP). However, the synthesis of gels by CRP techniques results in a different gelation process including a retarded gelation kinetic and higher swelling degrees of gels.[38] These higher swelling degrees are desired for applications in microfluidics where a pronounced stimuli response is needed. Additionally, it has been reported for CRP using RAFT agents that low ratios of chain transfer agent (CTA) to crosslinker are needed to obtain grafted gels instead of hyperbranched polymers.[39,40]

Here in this study we are interested in the preparation of temperature and pH-responsive hydrogels for application in microfluidics. The chosen approach allows to control the gels architecture and the segment length of two types of polymers incorporated into the gels and therefore, it provides the following advantages: (1) preparation of gels based on NIPAAm and a second responsive monomer, 4-vinylbenzoic acid (VBA), without losing temperature response while pH response is introduced, (2) improved swelling kinetics and (3) a pronounced stimuli response associated with high swelling ratio and thus high volume change. Due to the choice of grafted hydrogels, not only pH but also salt and solvent responsivity has been introduced. To our knowledge, this is the first report of a temperature-, pH-, solvent- and salt-sensitive graft copolymer gel. This gel is composed of a PNIPAAm backbone and PVBA graft chains. In order to obtain graft copolymer gels, an acrylamide functionalized macromonomer is synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization and Cu-catalysed alkyne and azide 1,3-dipolar cycloaddition. Furthermore, we report on the preparation of *net*-PNIPAAm-*g*-PVBA hydrogels with different grafting density, their detailed study with regard to swelling behaviour with respect to pH, temperature, solvent and salt concentration and we compare this behaviour with pure *net*-PNIPAAm hydrogel.

Experimental

Characterization techniques

^{1}H and ^{13}C NMR spectra were recorded using Bruker Avance III 500 spectrometer operating at 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C), with $CDCl_3$ or DMSO-d_6 as solvent at room temperature.

MALDI-TOF mass spectra were recorded on an Autoflex Speed MALDI-TOF-/TOF-TOF-System (Bruker, USA) in reflector mode with a smart beam laser (modified Nd:YAG laser). Solutions of the analyte were prepared in THF (1 mg ml^{-1}) and dithranol was used as the matrix (10 mg ml^{-1} in THF). Equal amounts of both solutions were mixed and spotted onto the MALDI plate.

IR analysis were recorded using a Vertex 80v (Bruker) with a DTGS-Detector. Samples were mixed with KBr (2 mg sample per 400 mg KBr), milled, and prepared as pellets. The spectra were recorded in the middle infrared spectroscopic range 4000–400 cm^{-1} with 2 cm^{-1} resolution. Thirty-two scans were added to every spectrum.

Differential scanning calorimetry (DSC) was performed on a DSC Q2000 (TA-Instruments) under N_2 in the range 5–50 °C at a heating rate of 1 °C min^{-1} as cycles consisting of 1st heating–cooling-2nd heating scans. The VPTT of the hydrogels was determined by averaging the maximum of the endothermic transition peak in the DSC plot.

Size-exclusion chromatography (SEC) measurements were carried out on a PL-GPC 50 Plus (Polymer Laboratories, USA) normal-temperature size exclusion chromatograph, equipped with refractive index detector and one column ResiPore (Agilent Technologies, USA) and on a second system GPC Series 1200 (Agilent Technologies, USA) with refractive index detector and one column PL MIXED-C (Agilent Technologies, USA). THF was used as eluent and the flow rate was 1 ml min^{-1}. Dispersity ($Đ = M_w/M_n$) of obtained polymers was determined based on calibration with polystyrene standards obtained from Polymer Standards Service (PSS, Germany). We converted the poly(4-vinylbenzoic acid) to poly(methyl-4-vinylbenzoate) *via* methylation by MeOH, dicyclohexylcarbodiimide (DCC) and 4-dimethylamino-pyridine (DMAP) before conducting the SEC measurement.

Materials

All chemicals were obtained from Sigma-Aldrich Chemical Co., NIPAAm was purified by recrystallization from *n*-hexane, and vacuum dried. All other chemicals were used as received.

***N*-(Prop-2-yn-1-yl)acrylamide (3).** To a 100 ml Schlenk-style, long-neck round-bottom flask equipped with a magnetic stirrer prop-2-yne-1-amine (**1**) (1.28 ml, 20 mmol) and 20 ml of dichloromethane (DCM) were added. The solution was sealed under argon atmosphere and submerged in an ice bath. In a separate vessel under argon atmosphere, acryloyl chloride (**2**) (2.5 ml, 27 mmol) was dissolved in 10 ml DCM, and the resulting solution was slowly added to the long-neck round-bottom flask. Thus the reaction was carried out at r.t. for 3 d. The solution was then added to 20 ml water, filtered, and extracted with diethyl ether (4 × 40 ml). The organic layer was dried with potassium sulfate, filtered, and then stripped of solvent under reduced pressure. The crude product was eluted through a silica gel column using *n*-hexane/ethyl acetate (40 : 60 v/v) to yield **3** as a white-yellow solid, yield 1.5 g (67%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.2 (t, $J = 2.8$ Hz, 1H, CH), 4.1 (dd, $J = 5.4$, 2.5 Hz, 2H; CH_2), 5.7 (dd, $J = 10.5$, 1.6 Hz, 1H; CH_2), 6.0 (br. s., 1H; NH), 6.1 (dd, $J = 16.8$, 10.3 Hz, 1H; CH), 6.3 (dd, $J = 16.9$, 1.6 Hz, 1H; CH_2). ^{13}C NMR (126 MHz, $CDCl_3$, δ): 29.18 (CH_2), 71.58 (CH), 79.31 (C), 127.08 (CH_2), 130.18 (CH), 165.28 (C═O).

3-Azido-1-propylamine (5). To a 250 ml one-neck round-bottom equipped with a magnetic stirrer and reflux condenser

3-chloropropyl-1-amine hydrochloride (**4**) (8.2 g, 63.1 mmol), sodium azide (12.6 g, 193.8 mmol) and 50 ml of water were added. The reaction was carried out for 24 h at 80 °C. The resulting solution was basified with sodium hydroxide and extracted with diethyl ether (4 × 40 ml). The organic layer was dried with potassium sulfate, filtered, and then stripped of solvent under a reduced pressure. Compound **5** was obtained as volatile, colourless oil, yield 4.1 g (65%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 3.39 (t, J = 6.7 Hz, 2H; CH_2), 2.82 (t, J = 6.8 Hz, 2H; CH_2), 1.74 (m, 2H; CH_2), 1.26 (br. s., 2H; NH_2).

DTP-N_3. To a 50 ml one-neck round-bottom flask equipped with a magnetic stirrer 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DTP) (915 mg, 2.51 mmol), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) (1470 mg, 2.83 mmol) and 8 ml of DCM were added. The solution was stirred at r.t. for 2 h. In a separate reaction vessel 5 (250 mg, 2.5 mmol) and *N*,*N*′-diisopropylethylamine (DIPEA) (797 mg, 6.17 mmol) was added to 2 ml of DCM. Subsequently, the resulting mixture was added to the one-neck round-bottom flask and stirred at r.t. for 3 d. The solution was then stripped of solvent under reduced pressure and the crude product was then eluted through a silica gel column using *n*-hexane/ethyl acetate (80 : 20 v/v) to yield DTP-N_3 as a yellow solid, yield 870 mg (78%). ^{1}H NMR (500 MHz, DMSO, δ): 0.86 (t, J = 6.8 Hz, 3H; CH_3), 1.2–1.4 (m, 18H; C_9H_{18}), 1.5–1.7 (m, 10H; C_2H_4 and $2CH_3$), 3.09 (q, 2H; CH_2), 1.8 (quin, J = 6.5 Hz, 2H; CH_2), 3.2–3.4 (m, 4H; 2 × CH_2), 7.89 (t, 1H; NH). ^{13}C NMR (126 MHz, $CDCl_3$, δ): 14.07 (CH_3), 22.65 (CH_2), 25.85 ($2CH_3$), 27.74 (CH_2), 28.92 (CH_2), 29.07 (CH_2), 29.30 (CH_2), 29.41 (CH_2), 29.51 (CH_2), 29.59 (CH_2), 29.60 ($2CH_2$), 31.88 (CH_2), 37.12 (CH_2), 37.93 (CH_2), 49.53 (CH_2), 57.16 (C), 172.64 (C═O), 220.31 (C═S).

PVBA-N_3. To a 10 ml Schlenk-style, long-neck round-bottom flask equipped with a magnetic stirrer DTP-N_3 (150 mg, 0.34 mmol), 4-vinylbenzoic acid (2.43 g, 16.4 mmol), 4,4′-azobis(4-cyanovaleric acid) (ACP) (4.2 mg, 0.015 mmol) and 5.5 ml of DMF were added. The solution was then subjected to three freeze–pump–thaw cycles, sealed under argon atmosphere, and submerged in an oil bath maintained at 80 °C. After 4 h the reaction was exposed to oxygen and quenched in liquid nitrogen. The solution was stripped of solvent under reduced pressure; the crude product was then dissolved in THF/MeOH (50 : 50 v/v) and precipitated in toluene to yield PVBA-N_3 as a yellow solid. M_n = 4500 g mol^{-1} (54%), $Đ$ = 1.4, ^{1}H NMR (500 MHz, DMSO-d_6, δ): 0.6–0.8 (t, 3H), 1.05–2.3 (m, 3H), 6.3–7.3 (m, 2H), 7.3–8.0 (m, 2H), 12.0–13.5 (m, 1H).

PVBA-AAm. To a 10 ml one-neck round-bottom flask equipped with a magnetic stirrer PVBA-N_3 (200 mg, 0.044 mmol), **3** (7 mg, 0.064 mmol) and 1.5 ml of DMF were added. In a second reaction vessel, $CuSO_4 \cdot 5H_2O$ (1.1 mg, 0.0044 mmol) and sodium ascorbate (0.88 mg, 0.0044 mmol) were dissolved in 150 μl water, respectively, and the resulting mixtures were added to the one-neck round-bottom flask. The reaction was carried out at r.t. for 3 d. Subsequently, the solution was stripped of solvent under reduced pressure; the crude product was then dissolved in THF/MeOH (50 : 50 v/v) and precipitated in *n*-hexane/ethyl acetate (60 : 40 v/v). The polymer was dialyzed (MWCO = 1000 Da) in water for 3 days and freeze-dried to yield PVBA-AAm as a yellow solid, yield 175 mg (86%). ^{1}H NMR (500 MHz, DMSO-d_6, δ): 0.6–0.8 (t, 3H); 1.05–2.3 (m, 3H); 4.15 (m, 2H); 4.36 (m, 2H); 2.28 (s, 6H); 5.58 (d, 1H); 6.10 (d, 1H); 6.24 (dd, 1H); 6.3–7.3 (m, 2H); 7.3–8.0 (m, 2H); 12.0–13.5 (m, 1H).

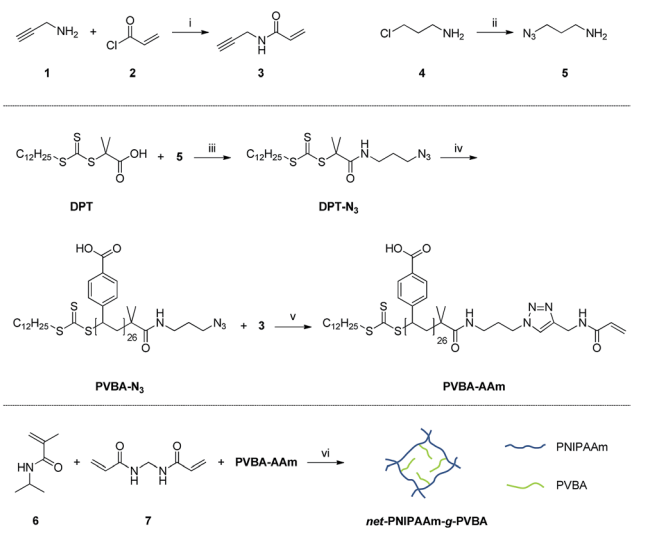
Hydrogel synthesis. To a 5 ml one-neck round-bottom flask equipped with a magnetic stirrer NIPAAm (141.5 mg; 1.25 mmol), *N*,*N*′-methylenebisacrylamide (BIS) (2.89 mg, 0.0186 mmol), azobisisobutyronitrile (AIBN) (2.05 mg, 0.0125 mmol) and 1 ml of pyridine were added. The PVBA-AAm content was varied as follows: (1) 0 mg (0 mmol); (2) 15.5 mg (0.0031 mmol); (3) 31 mg (0.0063 mmol); (4) 62 mg (0.0125 mmol). The resulting solution was subjected to three freeze–pump–thaw cycles, transferred into a glass tube of 3 mm diameter, sealed under argon atmosphere, and submerged in an oil bath maintained at 70 °C. The reaction was carried out for 24 h. Subsequently, the gel was separated from the glass tube and washed several times with water.

Results and discussion

Synthesis

The aim of this study is to prepare multi-responsive hydrogels based on graft copolymer gels. For this reason, an acrylamide end-functionalized poly(4-vinylbenzoic acid) macromonomer, PVBA-AAm, was synthesized. This was done by a combination of RAFT and click chemistry, a well-known approach for preparing polymers with high end-group functionalization (Scheme 1).[41]

The synthetic approach of the macromonomer designed for the fabrication of graft copolymer gels is shown in Scheme 1. Azide bearing RAFT agents have been widely studied in the context of post-polymerization functionalization. For example,



Scheme 1 Preparation of *N*-(prop-2-yn-1-yl)acrylamide (**3**), PVBA-AAm and *net*-PNIPAAm-*g*-PVBA hydrogels. Reagents and conditions: (i): NEt_3, DCM, 0 °C to r.t., 3 d, 67%. (ii) NaN_3, H_2O, 80 °C, 1 d, 65%. (iii) PyBOP, DIPEA, DCM, r.t., 78%. (iv) VBA, ACP, DMF, 80 °C, 4 h, Ar, 54%. (v) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF/H_2O (5 : 1), r.t., 3 d, 86%. (vi) AIBN, pyridine, 70 °C, Ar, 1 d.

Chen *et al.* and Sumerlin *et al.* reported the functionalization of DTP with 3-azidopropan-1-ol.[22,42–44] For our purposes, we selected to use 3-azidopropan-1-amine (**5**) (Scheme 1) due to the fact that amide bonds appear to be generally more stable to hydrolysis than ester bonds.[45] This is an important requirement for long-term stability of pH sensitive hydrogels in microfluidic platforms. The precursor **5** was prepared according to Pike *et al.* ^{1}H NMR study of 5 outlined the requested patterns as found in literature.[46] Then, the functionalization of DTP with **5** was examined to obtain the azide bearing DPT-N_3. It should be noted that trithiocarbonates rapidly undergo aminolysis with primary or secondary amines to form thiols and subsequently dithiocarbamates.[47,48] In fact, the functionalization of DPT was attempted by using a standard Steglich approach with DCC and DMAP.[49] The corresponding results indicated a high degradation of the DPT and low yields (>25%) of the desired DPT-N_3 (Scheme 1). Aiming for an increase of conversion, a more reactive coupling agent, PyBOP with DIPEA, was used (Scheme 1). Indeed, high yields up to 75% were obtained with this coupling agent. The ^{1}H NMR spectrum of DPT-N_3 clearly shows the propyl protons of the azide group at 3.30–3.40 ppm (Fig. 1-top (A)). Further FTIR analysis of DPT-N_3 confirmed the presence of an additional IR band corresponding to the azide stretch at 2100 cm^{-1} (Fig. 1-bottom (A)).

A homopolymer of 4-vinylbenzoic acid (VBA) PVBA-N_3 was subsequently prepared under standard RAFT conditions employing the prepared DTP-N_3 in conjunction with ACP as initiator in DMF at 80 °C (Scheme 1). With this a quantitative conversion of 6500 g mol^{-1} was attempted (conversion 54%). Initially, the polymerization initiated by DPT-N_3 was carried out with acrylic acid (AA) as monomer, well-known for its good pH-responsive in hydrogels. However, the polymerization of AA was associated by a degradation of the azide end-group. It has been reported that azides undergo 1,3-cycloaddition with electron-poor olefins at high temperatures and long reaction time as side reaction.[50,51] In order to circumvent this latter side reaction and to preserve high end group functionalization of DPT-N_3, VBA was used. FTIR analysis showed no side reaction of VBA with the azide group at the given polymerization conditions (for detailed information see ESI†). The PVBA-N_3 homopolymer was further characterized by SEC, FTIR spectroscopy, ^{1}H NMR spectroscopy and MALDI TOF mass spectrometry (MALDI TOF MS). The SEC measurements revealed a moderate dispersity indicating that the polymerization was controlled (M_w/M_n = 1.4). Note that for the SEC measurement the carboxylic acid group of PVBA-N_3 was methylated to suppress interactions with the column material. Retention of the terminal azide groups was confirmed by the presence of the azide stretch at 2100 cm^{-1} (Fig. 1-bottom (B)). The corresponding peaks of PVBA-N_3 were identified by the structure assignment of PVBA-N_3 in the ^{1}H NMR spectrum (Fig. 1-top (B)). Furthermore, the absolute molar mass was calculated to be 4500 g mol^{-1} by end-group analysis. This value is in good consistence of an average molar mass of 4400 g mol^{-1} determined by MALDI TOF MS (Fig. 2, ESI†). Moreover, the difference in mass between the major peaks was calculated to be 148 g mol^{-1}. This confirms that VBA is the repeating unit in PVBA-N_3 (repeat unit mass = 148.05 g mol^{-1}).

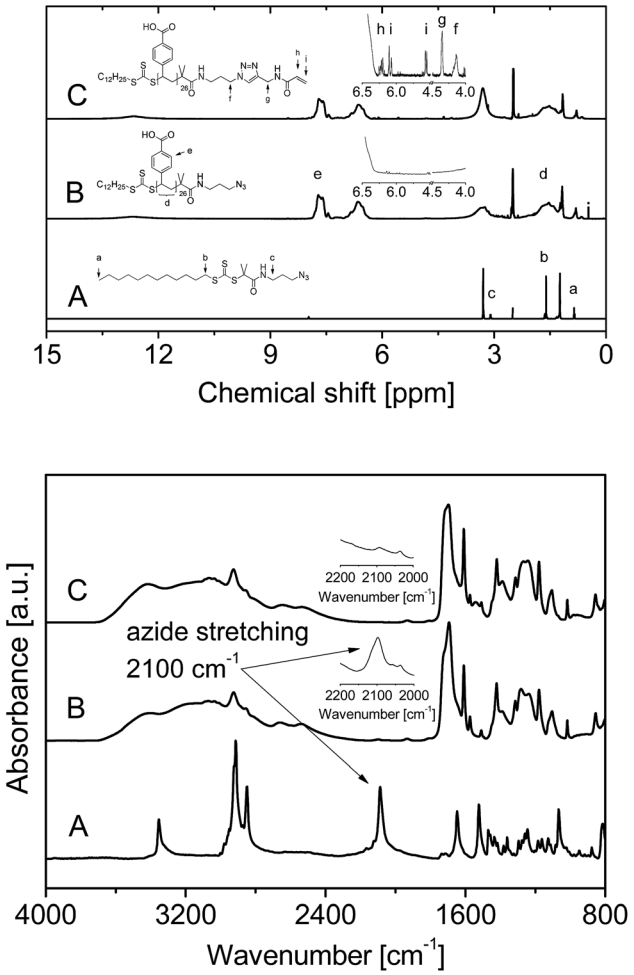


Fig. 1 ^{1}H NMR (top) and FTIR (bottom) spectrum of the functionalized DPT-N_3 (A), the azide bearing polymer PVBA-N_3 (B) and the acrylamide containing macromonomer PVBA-AAm (C).

To prepare the final PVBA-AAm macromonomer, an acrylamide, *N*-(prop-2-yn-1-yl)acrylamide **3**, was synthesized (Scheme 1). After testing the efficiency of the click reaction with a variety of Cu sources (CuBr, CuI and $CuSO_4$/sodium ascorbate), ligands (DIPEA and PMEDTA), and solvents (DMF, DMF/H_2O and THF), ^{1}H NMR studies indicated that the catalyst $CuSO_4$ in conjunction with sodium ascorbate in DMF/H_2O gives the best results obtaining reaction yields close to completion. In the resulting ^{1}H NMR spectrum (Fig. 3, ESI,† magnified spectrum), the appearance of the proton signals at 5.58 and 6.11 ppm, attributed to CH_2 of the acrylamide unit, revealed the effective conversion of PVBA-N_3 with **3** (Scheme 1). This indicates the formation of the triazole ring on the polymer PVBA-N_3 upon click reaction (Fig. 1-top (C)). Additionally, IR experiments showed the complete disappearance of the azide stretch at 2100 cm^{-1} (Fig. 1-bottom (C)).

Hydrogel synthesis and characterization

Finally, a series of grafted PNIPAAm gels containing 0.25 to 1 mol% PVBA-AAm were synthesized in pyridine with 1.5 mol% BIS as crosslinker and 1 mol% AIBN as initiator (Entry 2–4 in

Table 1 Composition and VPTT of the prepared *net*-PNIPAAm (Entry 1) and *net*-PNIPAAm-*g*-PVBA (Entry 2–4) hydrogels

		Feed composition				Molar composition[a]		
Entry	Sample name	NIPAAm [mmol]	PVBA-AAm [μmol]	BIS [μmol]	AIBN [μmol]	NIPAAm	VBA	VPTT[b] [°C]
1	0.00% PVBA	1.25	0.00	18.6	12.5	100	0	32.4
2	0.25% PVBA	1.25	3.13	18.6	12.5	96	4	33.0
3	0.50% PVBA	1.25	6.25	18.6	12.5	89	11	33.2
4	1.00% PVBA	1.25	12.5	18.6	12.5	82	18	33.3

[a] Composition in the prepared hydrogel determined by FTIR, for detailed information see ESI. [b] Determined in water by DSC.

Table 1). Low grafting density of PVBA-AAm in the gels resulted in the desired sharp VPTT of the PNIPAAm network. The network structure thus obtained is indicated in Scheme 1: the network is formed by a slightly crosslinked PNIPAAm gel structure with a few free tangling chains made from PVBA-AAm of about M_n = 4500 g mol^{-1}. This allows for retaining the physical interactions of two homopolymers.

The hydrogel synthesized with PVBA-AAm are summarized in Table 1. The networks formed were classified as *net*-PNIPAAm-*g*-PVBA hydrogel (Entry 2–4 in Table 1). A pure *net*-PNIPAAm hydrogel was used as a model gel (Entry 1 in Table 1). The determined molar composition of NIPAAm and VBA calculated by FTIR spectroscopy is in good agreement with the initial feed compositions (for further information see ESI†). Importantly, all prepared gels were washed several times with water to remove unreacted monomer as well as organic solvent before conducting stimuli response studies. Equilibrium swelling properties of the graft copolymer gels were studied at various temperatures (20–50 °C), pH values (2–10), solvent contents (0–100%) and salt concentrations (0–1 mol l^{-1}). Thus, hydrogel disks 5 mm in diameter were placed in solution and equilibrated for 1 d. Samples were removed from solution, blotted with filter paper to remove excess water on the surface and weighed. The swelling degree Q_w, was calculated for the hydrogels using the following equation:

$$Q_w = \frac{W_s - W_d}{W_d} \quad (1)$$

where W_s is the weight of the swollen hydrogel and W_d is the weight of the dried gel. Equilibrium swelling studies indicated that the prepared graft copolymer gels are capable of responding to environmental pH, temperature, organic solvent and salt. The basic principle of the temperature and pH sensitivity of *net*-PNIPAAm-*g*-PVBA hydrogels is illustrated in Fig. 2A. In total, 4 different swelling states can be addressed depending on the temperature and the pH value.

pH response. Fig. 3 shows the influence on the swelling degree at r.t. (A) and 50 °C (C). The pH dependency was

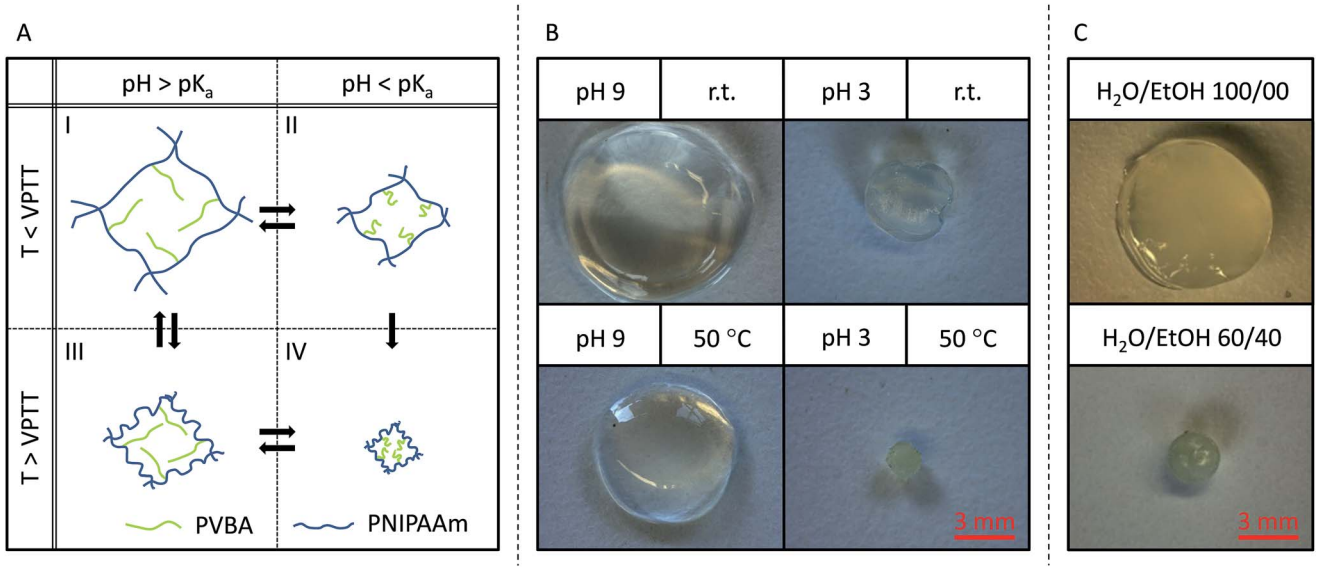


Fig. 2 (A) Schematic illustration of the temperature and pH response of *net*-PNIPAAm-*g*-PVBA hydrogels: (I) PNIPAAm and PVBA swollen; (II) PNIPAAm swollen, PVBA collapsed; (III) PNIPAAm collapsed, PVBA swollen; (IV) PNIPAAm and PVBA collapsed. (B) A *net*-PNIPAAm-*g*-PVBA hydrogel containing 1 mol% PVBA-AAm at different conditions: hydrogel totally swollen (pH 9, r.t.); hydrogel partially collapsed (pH 9, r.t. and pH 9, 50 °C); hydrogel completely collapsed (pH 3, 50 °C). (C) A *net*-PNIPAAm-*g*-PVBA hydrogel containing 0.25 mol% PVBA-AAm at different solvent mixtures at r.t.: hydrogel totally swollen (H_2O/EtOH 100%/0%); hydrogel completely collapsed (H_2O/EtOH 60%/40%).

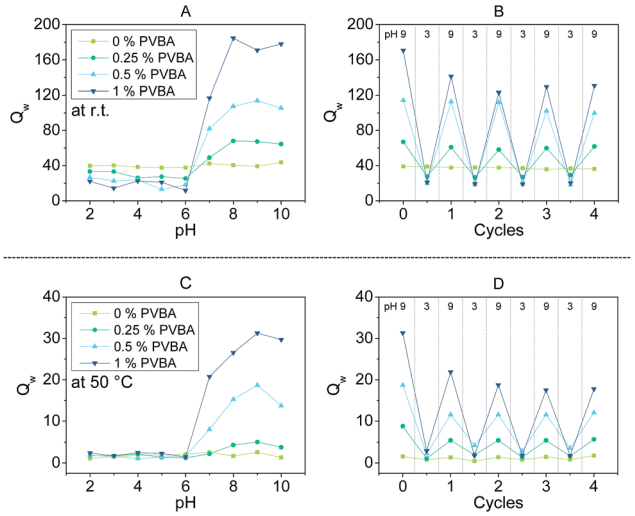


Fig. 3 Equilibrium swelling behaviour as a function of pH at r.t. (A) and 50 °C (C) in pH buffer solution and oscillatory swelling behaviour as a function of pH at r.t. (B) and 50 °C (D) for pure *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels.

investigated in the range of pH values from 2 to 10 for two cases: one was at a temperature below (T < VPTT, PNIPAAm swollen) while the other was above the VPTT of PNIPAAm (T > VPTT, PNIPAAm collapsed). For both, pure *net*-PNIPAAm hydrogel exhibits a nearly constant swelling degree of about 40 at r.t. and 2 at 50 °C independent on the pH value. Considerably high stimuli response is already observed with low molar amount of PVBA-AAm between 0.25 and 1 mol%. The swelling curves of *net*-PNIPAAm-*g*-PVBA hydrogels at r.t. and 50 °C show a sharp increase in swelling from pH 6 to 8, near the pK_a 7.1 of VBA homopolymers.[52] At pH values above and below the transition from pH 6 to 8, the swelling degree is almost consistent. This pH response is caused by the carboxylic acid groups of the PVBA grafts. They are ionized at pH values above 6, resulting in large osmotic swelling force associated with significantly swelling of the hydrogel. Furthermore, the pH response increases with increasing PVBA content. This behavior is independent whether the PNIPAAm backbone is swollen at r.t. or collapsed at 50 °C. For example, the change in swelling of *net*-PNIPAAm-*g*-PVBA at r.t. between pH 3 and pH 9 increases from 40, over 90 to 160. Importantly, the pH response of *net*-PNIPAAm-*g*-PVBA at r.t. is higher than at 50 °C due to the collapsed state of PNIPAAm at temperatures above the VPTT.

For technical use, for instance as a chemomechanical valve, the swelling/deswelling behavior should be consistent and therefore the reversibility was tested by repeated cycling between buffers at pH 3 and pH 9. The experiment was conducted at r.t. (Fig. 3B) and 50 °C (Fig. 3D) to investigate the impact of PNIPAAm backbone. Naturally, pure *net*-PNIPAAm hydrogels exhibit no appreciable change in swelling across several cycles independent on the temperature. On the contrary, reversible swelling/deswelling response is detected for the graft copolymer gels at r.t. and 50 °C. It is notable that *net*-PNIPAAm-*g*-PVBA shows a substantially reduced degree of swelling after the first cycle. This behavior is more pronounced for hydrogels with higher grafting density. For the *net*-PNIPAAm-*g*-PVBA hydrogel containing 1 mol% PVBA-AAm a drop of the swelling degree after the first cycle at r.t. from 171 to 141 is observed, whereas the *net*-PNIPAAm-*g*-PVBA hydrogel containing 0.25 mol% PVBA-AAm shows a drop from 67 to 61. These swelling degrees stabilize over the remaining 3 cycles. We assume that this phenomenon is caused by the buffer changing. This leads to higher ionic strength over various cycles resulting in lower swelling degrees. Regardless, the *net*-PNIPAAm-*g*-PVBA hydrogels show a reliable pH response with high volume different upon multiple pH changes.

Temperature response. As done for the pH response, the temperature behavior was investigated for two cases: at a pH above (pK_a < pH, PVBA swollen) and below the pK_a of VBA (pK_a > pH, PVBA collapsed). The temperature dependence at pH 9 of *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels is shown in Fig. 4A. The *net*-PNIPAAm hydrogel shows the typically VPTT between 30 and 35 °C. This value is in good agreement with 32.4 °C determined by differential scanning calorimetry DSC (Fig. 5-left). The VPTT of the graft copolymer gels is in the same range from 30 to 35 °C. DSC measurements further confirm these data and display a slight shift of the VPTT to higher values from 32.4 to 33.3 °C (Table 1). The degree of deswelling is significantly affected by the PVBA content. Graft copolymer gels with high PVBA content undergo higher shrinkage compared to gels with lower PVBA content. For example, pure *net*-PNIPAAm shows a change in swelling from 51 to 1, whereas *net*-PNIPAAm-*g*-PVBA containing 1 mol% PVBA-AAm exhibits a change in swelling from 204 to 19. Additionally, all *net*-PNIPAAm-*g*-PVBA hydrogels show a sharp volume phase transition indicated by a high drop of swelling between 30 and 35 °C. Turbidity experiments of *net*-PNIPAAm-*g*-PVBA containing 1 mol% PVBA-AAm further confirmed a sharp transition similar to pure *net*-PNIPAAm (Fig. 5-right). This behavior is particularly beneficial from the

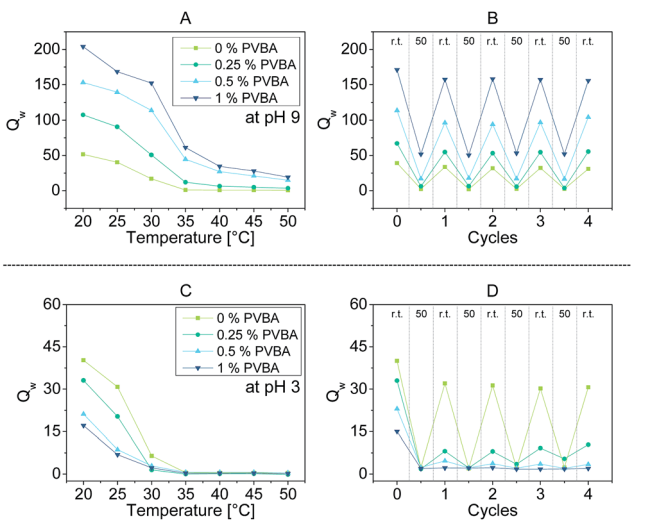


Fig. 4 Equilibrium swelling behaviour as a function of temperature at pH 9 (A) and pH 3 (C) and oscillatory swelling behaviour as a function of temperature at pH 9 (B) and pH 3 (D) for pure *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels.

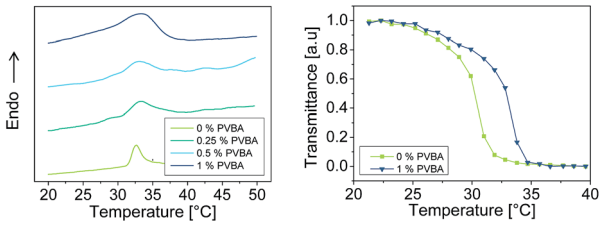


Fig. 5 VPTT determination of pure *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels by DSC (left) and UV/Vis light transmittance (right) determined in water.

application point of view, because small temperature changes already lead to high stimuli response.

In contrast, the VPTT at pH 3 for *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels is shifted towards lower temperatures below 30 °C (Fig. 3C). It has been shown that the LCST of PNIPAAm and the VPTT of *net*-PNIPAAm hydrogels is highly affected by salts and their concentrations.[23,53–56] For this reason, we suspect that this shift is provoked by the salt of the buffer solution. Furthermore, the deswelling of the graft copolymer gels at pH 3 is less pronounced compared to the *net*-PNIPAAm hydrogel. As more PVBA grafts are incorporated into the gel, the hydrophobicity of the gel increases when these graft are protonated. The resulting hydrophobicity at pH 3 is associated with a significant lower swelling degree. Oscillatory swelling experiments were also performed to investigate whether the responses to the environmental temperature is reversible. Fig. 4 shows the repeated cycling between r.t. and 50 °C at pH 9 (B) and pH 3 (D). As it could be expected, the deswelling and swelling cycles at pH 9 are reproducible at least for four times of all investigated hydrogels. Here, the swelling/deswelling response is more pronounced with increased PVBA content. In contrast to pH 9, *net*-PNIPAAm-*g*-PVBA hydrogels with higher PVBA content exhibit at pH 3 no repeated swelling/deswelling behavior whereas for the *net*-PNIPAAm hydrogel, a reversible swelling/deswelling response is detected. We propose that this phenomenon is caused by the formation of intrachain complexes by hydrogen bonds between the amide groups of the PNIPAAm backbone and the protonated carboxylic groups of the graft chains as well as between carboxylic groups of single graft chains (Fig. 6). Moreover, π-interactions are also possible due to increased spatial proximity of the collapsed graft chains. These interactions act as diffusion barrier and decrease the accessibility of water to the NIPAAm-units. As a consequence, the temperature stimulus of *net*-PNIPAAm-*g*-PVBA hydrogels at pH 3 is not suitable for multiple uses. However, further research is needed for full understanding.

Solvent and salt response. It is known that *net*-PNIPAAm hydrogels undergo swelling/deswelling at different water/solvent compositions.[14,57] For this reason, we were particularly interested in the swelling behavior of the *net*-PNIPAAm-*g*-PVBA hydrogels in different solvent compositions. As shown in Fig. 7A, the model systems acetone/water was used and the composition of the solvent mixture was varied. Note that beside acetone/water further alcohol/water mixtures were tested (Fig. 6,

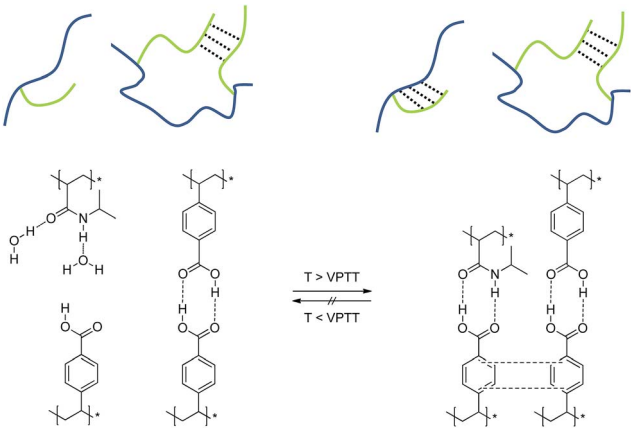


Fig. 6 Possible π-interactions and H-bonded complexes between the CONH-groups of the PNIPAAm backbone and the COOH-groups of the PVBA graft chains as well as between COOH-groups of different PVBA graft chains at pH 3, leading to a reduced repeated swelling.

ESI†). The *net*-PNIPAAm and *net*-PNIPAAm-*g*-PVBA hydrogels are totally swollen at r.t. in pure water. As expected, the swelling degree increases with increasing PVBA-content. Furthermore, the hydrogels exhibit a significant higher swelling degree in pure water than in buffer solution caused by the lower ionic strength. At about 20 vol% acetone, all hydrogels collapse and start to swell again at about 60 vol% acetone. At higher acetone content, between 70 and 90 vol%, higher swelling of the *net*-PNIPAAm-*g*-PVBA compared to pure PNIPAAm is observed. This may be explained by a good solubility of the PVBA grafts at these water/acetone compositions. In any case, *net*-PNIPAAm-*g*-PVBA hydrogels respond to different water/acetone mixtures almost similar like the *net*-PNIPAAm hydrogel.

In addition to solvent response, the salt response of *net*-PNIPAAm-*g*-PVBA hydrogels was also investigated. It was reported that high salt concentration leads to partial deswelling of *net*-PNIPAAm hydrogels.[58] On the contrary, Satoh *et al.* reported from high salt resistivity of *net*-PVBA hydrogels compared to *net*-PAA hydrogels.[59] They supposed a stabilization of hydrogen-bonding hydrations of PVBA through ionic hydrations. For this reason, we were particularly interested in our *net*-PNIPAAm-*g*-PVBA hydrogels behavior and comparing it with *net*-PNIPAAm and *net*-PVBA hydrogels. The effect of the salt concentration of

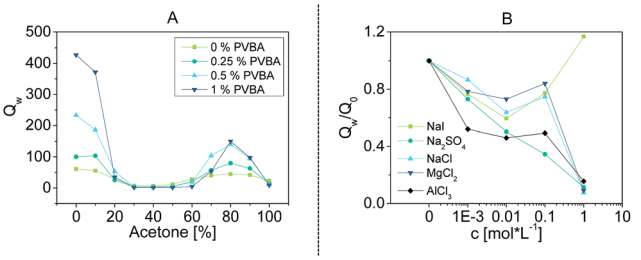


Fig. 7 Equilibrium swelling behaviour as a function of acetone concentration at r.t. (A). Change in swelling as function of various salts at different concentrations for *net*-PNIPAAm-*g*-PVBA containing 0.5 mol% PVBA-AAm (B).

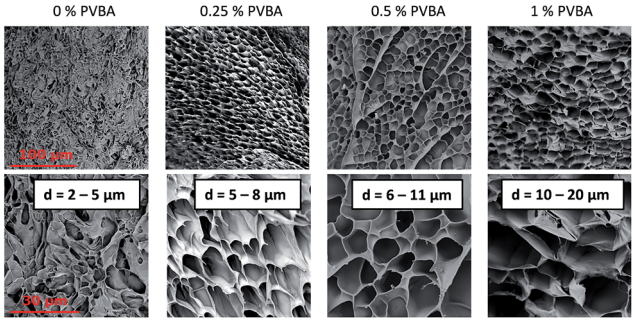


Fig. 8 SEM images of the freeze-dried hydrogels with average pore diameter. Scale bar 100 µm (top) and 30 µm (bottom).

various salts is presented in Fig. 7B. In this experiment we detect a decreased swelling degree of *net*-PNIPAAm-*g*-PVBA containing 0.5 mol% PVBA-AAm macromonomer with increasing salt concentration for all studied salts except NaI. This behavior is in good accordance with the results of a conventional *net*-PNIPAAm hydrogel (Fig. 5, ESI†). A high salt resistivity by the incorporated PVBA grafts chains is not detected or dominated by the deswelling of PNIPAAm with increased salt concentration. The incorporated molar amount of PVBA was low and its impact is comparatively small. We belief therefore that high salt resistivity might only be detected for a high content of PVBA.

Morphology studies. Fig. 8 shows the SEM studies of the swollen freeze-dried hydrogels. It is important to note that the observed honeycomb macropores are a result of the gradual growth of microstructural ice crystals during the freeze-drying process.[60,61] However, the results showed that the graft density of the PVBA-AAm macromonomer has a great influence on the morphology of the freeze-dried samples. Conventional *net*-PNIPAAm hydrogels exhibited smaller pore size compared to the graft copolymer gels. This is because the incorporation of ionic VBA increases the water content when the hydrogels are swollen. Thus, with increasing graft density the pore size increases, while the number of pores per unit area of the *net*-PNIPAAm-*g*-PVBA *versus* the pure *net*-PNIPAAm hydrogels in turn decreases. In addition, the *net*-PNIPAAm-*g*-PVBA hydrogels exhibit a more homogenous and dense architecture compared to pure *net*-PNIPAAm.

Conclusion

In summary, the synthesis of a PVBA-AAm macromonomer by RAFT polymerization and subsequent introduction of an acrylamide function *via* click chemistry was described. This functionalized macromonomer PVBA-AAm allowed gel formation of grafted copolymer gels. A series of temperature, pH, solvent and salt responsive graft copolymer gels composed of a PNIPAAm backbone and PVBA grafts were prepared. Low molar amount of PVBA-AAm from 0.25 to 1 mol% was sufficient to obtain high pH responsive hydrogels. Importantly, the incorporation of PVBA grafts did not influence the temperature transition of the PNIPAAm backbone. Moreover, the pH and temperature swelling/deswelling cycles were reversible. Solely the temperature oscillatory swelling experiments at pH 3 were not repeatable in all cases. A hypothesis about the formation of intrachain complexes by hydrogen bonds between amide groups and carboxylic groups as well as between carboxylic groups of different graft chains has been proposed to explain this phenomenon. Additionally, *net*-PNIPAAm-*g*-PVBA hydrogels exhibited a water/acetone and salt response similar to *net*-PNIPAAm hydrogels. These results show us the great potential suitably designed tetra-sensitive *net*-PNIPAAm-*g*-PVBA hydrogels as potential chemomechanical valve due to their specific ability to respond independently to four different environmental alterations with a sharp transition and a high volume change. In further studies, we will investigate the swelling kinetic and we will incorporate our graft copolymer gels in microfluidic platforms.

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