Tetra-Responsive Grafted Hydrogels for Flow Control in Microfluidics

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“Research is an organized method for keeping you reasonably dissatisfied with what you have.”

Charles F. Kettering
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Fundamentals and Aims
1 Theoretical Background

1.1 Free Radical Polymerization and Controlled Radical Polymerization

Radical polymerization is a process in which monomers form a polymer with radicals as the chain carriers.\(^1\) There are two groups of radical polymerization known as controlled radical polymerization (CRP) and free radical polymerization (FRP).

As shown in Figure 1.1, FRP comprises three key steps: (i) initiation, (ii) chain propagation (iii), and termination.\(^2\) The initiation step involves the formation of free radicals by an initiator. Typically, azo compounds and organic as well as inorganic peroxides are used. The initiating species adds monomer units to start the chain polymerization. The polymer chain propagates by the reaction between monomers and the active site on the polymer chain until low monomer concentration and radical termination by either combination (\(P_{n+m}\)) or disproportionation (\(P_n=\) and \(P_mH\)) occurs.\(^2\) FRP benefits from a simple production process accompanied by low costs and a wide range of compatible monomers.\(^3\) For this reason, FRP has become an important method to prepare polymers for everyday life, including polyethylene, polypropylene, polyvinyl chloride, and polystyrene. However, the high radical concentration during FRP leads to a poor control over the molar mass, the molar mass distribution, the composition, the architecture, and the functionality of the resulting polymer.\(^4\)

In order to overcome these drawbacks, various methods have been developed in which certain additives convert reversibly the chain carriers from an active into a temporary

<table>
<thead>
<tr>
<th>Initiation</th>
<th>(I_2 \rightarrow 2I^*)</th>
</tr>
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<tr>
<td>Chain propagation</td>
<td>(I^* + nM \rightarrow P_n^*)</td>
</tr>
<tr>
<td>Termination</td>
<td>(P_n^* + P_m^* \rightarrow P_{n+m})</td>
</tr>
<tr>
<td></td>
<td>(P_n^* + P_m \rightarrow P_n^{\text{eq}} + P_mH)</td>
</tr>
</tbody>
</table>

Figure 1.1: Mechanism of a free radical polymerization.\(^2\)
Theoretical Background

The most common methods are stable-radical-mediated polymerization (SRMP, e.g. nitroxide-mediated polymerization, organometallic-mediated radical polymerization), atom-transfer radical polymerization (ATRP), and reversible addition-fragmentation chain-transfer (RAFT) polymerization. Note that RAFT polymerization is based on a degenerative exchange process, whereas SRMP and ATRP undergo a reversible activation/deactivation mechanism. Figure 1.2 shows the principle of SRMP and ATRP in which an equilibrium between active and dormant polymer chains occurs. Monomers can be added in the active form (P\textsubscript{n}·), while the polymer chains in the dormant form (P\textsubscript{n}-X) are unable to propagate. Typically, there is a rapid interconversion between active and dormant form with only a minority of active species and a large fraction of inactive polymer chains. This results in an equal rate of propagation of all polymer chains accompanied by a narrow molar mass distribution. However, an irreversible chain termination still occurs in CRP resulting in non-propagating species (around 1 - 10 %). Importantly, the remaining polymer chains in the dormant state can be reactivated, which allows the formation of block copolymers.

![Equilibrium between dormant and active polymer chain in a controlled radical polymerization](image)

**Figure 1.2:** Equilibrium between dormant and active polymer chain in a controlled radical polymerization, such as ATRP or SRMP.

**Reversible Addition-Fragmentation Chain-Transfer Polymerization**

RAFT polymerization was first reported by the CSIRO group in 1998. Chiefari and colleagues showed that a conventional free radical polymerization becomes a controlled radical polymerization by adding a thiocarbonylthio chain transfer agent (CTA), also known as RAFT agent. Since then, RAFT polymerization has become of increasing importance over the last two decades due to high number of polymerizable monomers (e.g. acrylates, styrene, vinyl ester), precisely controlled structure of the obtained polymers (e.g. random, block, gradient, grafted and star copolymers) and wide variety of suitable solvents (e.g. organic and protic solvents, ionic liquids, supercritical carbon dioxide). As shown in Figure 1.3, the RAFT agent is composed of a C=S double bond, a stabilizing group Z, and a free radical leaving group R. While the free radical leaving group R must be able to reinitiate the polymerization, the Z group allows fine-tuning of the C=S double bond activity.
1.1 Free Radical Polymerization and Controlled Radical Polymerization

**Figure 1.3:** (top) General structure of the CTA and examples of RAFT agents. (bottom) Guideline for the selection of R and Z groups for various polymerizations. For R, fragmentation rates decrease from left to right. For Z, fragmentation rates increase and addition rates decrease from left to right. Dashed lines represent poor control over dispersity (MMA = methyl methacrylate, HPMAM = N-2-hydroxypropyl methacrylamide, St = styrene, MA = methyl acrylate, AM = acrylamide, AN = acrylonitrile, VAc = vinyl acetate, NVP = N-vinylpyrrolidone, NVC = N-vinylcarbazole).[6]

The following four classes of CTAs are mainly used for RAFT polymerization (Figure 1.3):[6]

1. Dithioesters are the best-known and most often employed class of CTAs developed by Rizzardo and colleagues.[5] Due to their high transfer constants, they are suitable for a wide range of monomers, such as styrene, acrylates, and methacrylates. Dithioesters usually result in red-fluorescent polymers, which indicates an active thiocarbonylthio
compound suitable for a subsequent polymerization. Unfortunately, dithioesters tend to retardation under high CTA concentrations and are susceptible to hydrolysis.

2. Trithiocarbonates have been introduced by the CSIRO group and can be used for “more activated” monomers (e.g. styrene, and many additional examples).\(^7\) Compared to dithioesters, trithiocarbonates show higher stability, tolerance to water, and result in yellow colored but less intensely fluorescent polymers. Furthermore, trithiocarbonates exhibit high transfer constants and cause less retardation.

3. Xanthate-mediated RAFT, also known as RAFT/MADIX (Macromolecular Design via the Interchange of Xanthates), is particularly effective with “less activated” vinyl esters as well as vinyl amides like vinyl acetate, \(N\)-vinylcarbazole, and \(N\)-vinylpyrrolidone.\(^8\)

4. Dithiocarbamates are suitable for electron-rich monomers (e.g. vinyl acetate, among others). The \(N\)-substituents determine the reactivity of the CTA. Importantly, dithiocarbamates allow the polymerization of monomers with highly dissimilar reactivities (e.g. styrene and vinyl acetate).\(^9,10\)

Figure 1.4: Examples of polymer compositions, architectures, and functionalities prepared by RAFT polymerization.\(^4\)

As shown in Figure 1.4, RAFT polymerization offers many opportunities for polymer design, including various compositions (e.g. homopolymers, random copolymers, gradient
1.1 Free Radical Polymerization and Controlled Radical Polymerization

copolymers, block copolymers, and graft copolymers), architectures (e.g. linear polymers, star/multi-arm polymers, comb/brush polymers, branched polymers, and polymer networks), and functionalities (e.g. end-functional, side-functional, multi-functional branched, macromonomers, and telechelic polymers).

Mechanism of Reversible Addition-Fragmentation Chain-Transfer Polymerization

Because it was planned in this work to synthesize the macromonomers by RAFT polymerization, it is needed to consider the reaction mechanisms. Figure 1.5 displays the generally accepted mechanism of RAFT polymerization with a thiocarbonylthio compound.\[6,11\] Compared to conventional free radical polymerization, two additional propagation equilibria occur besides initiation and termination. In the reversible chain transfer/propagation stage, the reaction of the propagating radical $P_n^-$ with the initial CTA (1) forms an intermediate radical (2). This radical fragments subsequently either back to the initial CTA (1) and $P_n^-$ or to the dormant polymeric thiocarbonylthio compound (3) and radical R'. Reinitiation of radical R' by reacting with monomer units results in a new propagating radical $P_m^-$. When the initial CTA is consumed, the chain equilibrium/propagation stage follows by a fast exchange between the dormant polymeric thiocarbonylthio compounds.

| Initiation | $l_2 \rightarrow z^i \xrightarrow{n^M} P_n^- $ |
| Reversible chain transfer/propagation | $P_n^- + S-Z-R \leftrightarrow P_n^- + S-Z-R \leftrightarrow P_n^- + S-Z+R' $ |
| Reinitiation | $R' \xrightarrow{M} R-M' \xrightarrow{n^M} P_m^- $ |
| Chain equilibrium/propagation | $P_n^- + S-Z-P_m \leftrightarrow P_n^- + S-Z-P_m \leftrightarrow P_n^- + S-Z+P_m^- $ |
| Termination | $P_n^- + P_m^- \rightarrow P_{n+m}^- \quad P_n^- + P_m^- \rightarrow P_n^- + P_m^H $ |

Figure 1.5: Mechanism of the reversible addition-fragmentation chain-transfer polymerization.\[6\]
(3 and 4) and the active propagating radicals (Pₙ and Pₘ). Because propagation for all chains is equally likely, polymers of narrow molecular weight distribution can be obtained. Termination by combination (Pₙ₊ₘ) or disproportionation (Pₙ⁻ and PₘH) finally stops the polymerization.

In order to perform a controlled RAFT polymerization, Rizzardo and colleagues proposed the following guideline:[6]

- A reactive C=S double bond exists in the initial CTA (1) as well as in the polymeric thiocarbonylthio compound (3)
- Rapid fragmentation of both intermediate radicals (2 and 5) without side reaction
- Preferred fragmentation of the intermediate radical (2) to the dormant polymeric thiocarbonylthio compound (3) and radical R·
- Efficient re-initiation by the free radical R·

### 1.2 Functional Polymers with Click Chemistry

The intentional and specific coupling of two single molecules by a “click” reaction represents a key element in organic chemistry and has been widely used for the functionalization of polymeric materials.[12,13] Sharpless and colleagues introduced the concept of “click chemistry” in 2001. According to Sharpless et al., a reaction has to fulfill the following criteria to be called “click” reaction:[14]

- modular and wide in scope
- very high yields
- minimal and inoffensive by-products (removable by nonchromatographic methods like crystallization or distillation)
- stereospecific (not necessarily enantioselective)
- simple reaction conditions (ideally insensitive to oxygen and water)
- readily available starting materials and reagents (no solvent, non-toxic solvent or the solvent should be easy to remove)
- simple product isolation
These characteristics are usually driven by a high thermodynamic driving force (> 20 kcal·mol⁻¹) resulting in a quick reaction close to completion with high selectivity for one product.\(^{[14]}\) Figure 1.6 shows a selection of reactions, which meet the criteria of “click chemistry”, including (i) copper-catalyzed azide-alkyne cycloaddition (CuAAC), (ii) strain-promoted azide-alkyne cycloaddition (SPAAC), (iii) Diels–Alder reaction, and (iv) thiol–ene reaction.\(^{[15]}\)

| Copper-catalyzed azide-alkyne cycloaddition | \[
R_1\equiv H + \overset{\mathrm{6}}{N=N=N}R_2 \xrightarrow{\text{Cu(I)}} \overset{\mathrm{N=N}}{N}R_1-N-R_2
\]
| Strain-promoted azide-alkyne cycloaddition | \[
\overset{\mathrm{8}}{R_1} \quad + \overset{\mathrm{6}}{N=N=N}R_2 \xrightarrow{\text{Cu(I)}} \overset{\mathrm{N=N}}{N}R_1-N-R_2
\]
| Diels-Alder reaction | \[
\overset{\mathrm{R_1}}{\equiv} \quad + \overset{\mathrm{R_2}}{\equiv} \quad \xrightarrow{} \overset{\mathrm{R_1}}{\equiv} \overset{\mathrm{R_2}}{\equiv}
\]
| Thiol-ene reaction | \[
\overset{\mathrm{R_1-S}}{\equiv} \quad + \overset{\mathrm{R_2}}{\equiv} \quad \xrightarrow{\text{1) Free radical}} \overset{\mathrm{R_1}}{\equiv} \overset{\mathrm{S}}{\equiv} \overset{\mathrm{R_2}}{\equiv}
\]

**Figure 1.6:** Selection of “click” reactions: (i) copper-catalyzed azide-alkyne cycloaddition, (ii) strain-promoted azide-alkyne cycloaddition, (iii) Diels–Alder reaction, and (iv) thiol–ene reaction.\(^{[15]}\)

**Copper-Catalyzed Azide-Alkyne Cycloaddition**

In 2002, CuAAC was independently discovered by Sharpless and Meldal.\(^{[16,17]}\) Both laboratories reported that the reaction of an organic azide with a terminal alkyne is significantly accelerated in the presence of Cu(I). Interestingly, although two different catalytic systems were tested by Sharpless and Meldal (CuSO\(_4\) with sodium ascorbate in water versus CuI with various ligands in organic solvent), excellent yields and high regioselectivity were achieved in both studies. Figure 1.6 shows the reaction between an azide and an alkyne catalyzed by Cu(I) to give a 1,4-disubstituted 1,2,3-triazol. It should be mentioned that the reaction without copper catalysis under elevated temperatures is over 10\(^7\) times slower and results in a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles.\(^{[15]}\)

CuAAC has become the most important representative of “click” reactions because it can be performed in protic as well as aprotic solvents and is compatible with most functional groups (no protecting group chemistry is needed). A wide range of different Cu(I) sources are available as well, including Cu(I) salts (e.g. chloride, bromide, iodide,
acetate) and Cu(I) complexes (e.g. \([\text{Cu(CH}_3\text{CN)}_4]\text{PF}_6, [\text{Cu(CH}_3\text{CN)}_4]\text{OTf}]\). In order to stabilize the Cu(I) catalyst and to promote the formation of copper(I)-acetylide, a variety of ligands (1-5 equivalents) have been employed. Important ligands are triethylamine, \(N,N\)-diisopropylethylamine (DIPEA), \(N,N,N',N'',N''\)-pentamethyldiethylenetriamine (PMDTA) or 2,2'-bipyridine. However, catalytically active Cu(I) tends to be oxidized to inactive Cu(II) or to disproportionate to Cu(0) and Cu(II). Additionally, Cu(II) ions promote the formation of diynes from two terminal alkynes as an undesired by-product (Glaser coupling, Eglington coupling). For this reason, CuCAA employing Cu(I) as the catalyst should be performed under protective gas with rigorous exclusion of oxygen. One possibility of circumventing oxygen-free conditions is to form the Cu(I) species \textit{in situ}. For example, Cu(I) can be generated by reducing a Cu(II) salt like CuSO\(_4\)·5H\(_2\)O or Cu(OAc)\(_2\) with sodium ascorbate as a mild reducing agent. Sodium ascorbate provides the additional advantage that oxygen is reduced and the formation of oxidative side products is avoided. An alternative method is the comproportionation reaction, where Cu(0) and Cu(II) form Cu(I) \textit{in situ}. However, this method suffers from prolonged reaction times.

**Mechanism of Copper-Catalyzed Azide-Alkyne Cycloaddition**

It is important to note that the exact mechanism of CuAAC is still unknown and further research is needed for full understanding. The early proposed mechanism of CuAAC based on mononuclear copper(I) acetylides complexes. However, density-functional theory studies and kinetic experiments indicated that bis(copper) complexes are involved throughout CuAAC.

![Figure 1.7: Mechanism of the copper-catalyzed azide-alkyne cycloaddition proposed by Bertrand and colleagues.][21]
Recently, Bertrand and colleagues reported of the isolation of 3,5-bis(copper) triazole from the CuAAC reaction employing cyclic (alkyl)(amino)carbenes.\[21\] Figure 1.7 displays their proposed mechanism of the CuAAC. Although the bis-copper complex is the kinetically preferred cycle, bis- as well as mono-copper acetylide complexes are involved in two individual catalytic pathways. In the first step, the terminal hydrogen is removed from alkyne \(7\) by a base to form a mono-copper acetylide \(8\). Subsequently, mono-copper acetylide \(8\) can undergo the slow catalytic cycle and reacts with the azide \(9\) to a mono(metallated) triazole \(10\). Demetallation forms the 1,4-disubstituted 1,2,3-triazole \(11\) using alkyne \(7\) as the proton source and regenerates the mono-copper acetylide complex \(8\). Alternatively, mono-copper acetylide \(8\) gives a \(\pi,\sigma\)-bis(copper) acetylide \(12\) with a second LCuX \(6\). The obtained bis(copper) acetylide \(12\) forms subsequently a bis(copper) triazole \(13\) with azide \(9\), which then undergoes demetallation with an alkyne \(7\) to produce the 1,4-disubstituted 1,2,3-triazole \(11\) and the \(\pi,\sigma\)-bis(copper) acetylide of type \(12\).

### Other “Click” Reactions

Beside CuAAC reaction, there are a few other reactions that meet the criteria for a “click” reaction: (i) SPAAC, (ii) Diels-Alder reaction, and (iii) thiol-ene reaction (Figure 1.6).\[15\]

SPAAC was first reported by Bertozzi and colleagues in 2004.\[22\] This method is based on a reaction of azides with highly strained alkynes called cyclooctynes. SPAAC has the advantage of a rapid and efficiently formed triazole product without using a Cu(I) catalyst, which is particularly interesting for chemical biology, where the cytotoxicity of copper prevents many applications. However, SPAAC suffers from the fact that cyclooctynes are often unstable, thus cyclooctynes need to be introduced at a late stage in the synthesis.\[23\] Furthermore, the resulting product is a mixture of 1,4- and 1,5-disubstituted 1,2,3-triazoles.\[15\]

Another example of “click” reactions is the Diels-Alder reaction, which was first described by Otto Diels and Kurt Alder in 1928.\[24\] The Diels-Alder reaction is classified as \([4+2]\) cycloaddition between an electron-rich diene (e.g. furan) and an electron-poor dienophile (e.g. maleic acid) to give a cyclohexene system (Figure 1.6).\[25\] Interestingly, Diels-Alder reactions can be thermally reversible named as a retro-Diels-Alder reaction. For instance, 1,3-cyclopentadiene readily forms \(endo\)-dicyclopentadiene via Diels-Alder reaction at room temperature, while \(endo\)-dicyclopentadiene undergoes a retro-Diels-Alder reaction when heated to 180 °C.\[26\] Besides the conventional Diels-Alder reaction, the concept of \([4+2]\) cycloadditions has been extended to other \(\pi\)-systems like carbonyls and imines to obtain heterocycles. The reaction using hetero-atoms is known as the hetero-Diels-Alder reaction.\[27\]
In 2007, Schlaad and colleagues first introduced the addition of mercaptans (R-SH) onto vinyl double bonds as a click reaction using a radical source as the catalyst (thiol-ene reaction).\cite{28} Thiol-ene reactions have the advantage that the radical source can be generated in different ways, including thermal, redox, and photochemical methodologies.\cite{15} Furthermore, the high reactivity of thiols usually results in rapid formation of the thiol-ene product. However, thiols can be readily oxidized to form disulfides.\cite{29}

**Combining Reversible Addition-Fragmentation Chain-Transfer Polymerization and Click Chemistry**

In the last decade, click chemistry has become increasingly important to modify polymers prepared by RAFT polymerization with desired functional groups. In 2010, Barner-Kowollik and colleagues summarized the most important requirements for click reactions with one or more polymeric reagents, including single reaction trajectory, chemoselective, wide in scope, modular, stable compounds, high yields, fast timescale, large-scale purification, and equimolarity.\cite{30} Figure 1.8 shows different strategies for combining click chemistry and RAFT polymerization: (i) clickable monomers, (ii) clickable chain transfer agents, (iii) and post-polymerization modification.\cite{31}

A common method to combine RAFT polymerization and click chemistry is to use clickable monomers, which allows a high fraction of clickable groups in the final polymer. Furthermore, the number of clickable units is adjustable when a second monomer without a clickable group is employed in a copolymerization. Naturally, clickable groups can be converted before (preclick) as well as after (postclick) the polymerization. However, many desired monomers with clickable groups are commercially unavailable and require additional synthetic work. Furthermore, several groups have shown that clickable groups can affect the polymerization mechanism.\cite{31} For example, Perrier and O’Reilly reported degradation of azide groups under standard RAFT polymerization conditions by electron-poor monomers at high temperatures and long reaction time.\cite{32,33} Moreover, alkyne groups for CuAAC interfere with propagating radicals and tend to cross-link due to chain transfer by hydrogen abstraction.\cite{34}

Clickable transfer agents are another strategy for combining RAFT polymerization and click chemistry. The clickable group can be converted before (preclick) and after (postclick) the polymerization. Naturally, the clickable group can be incorporated into the RAFT agent with the free radical leaving group R or with the stabilizing group Z. Because the stabilizing group Z is lost to a small extent from the polymer chain due to irreversible chain termination, the free radical leaving group R is preferred to incorporate the clickable unit.\cite{34} Unfortunately, RAFT agents with clickable functionalities are often
commercially unavailable and the fraction of clickable groups is limited to one group per RAFT agent.\cite{31} As mentioned for clickable monomers, clickable groups of the RAFT agent can undergo side reactions during the RAFT polymerization.\cite{32,33} It is notable that due to the lower fraction of clickable groups, protection is optional because side reactions occur to a lesser extent compared to clickable monomers.\cite{34} Interestingly, the thiocarbonyl thio moiety of the RAFT agent can undergo hetero Diels–Alder reactions. Barner-Kowollik and colleagues reported hetero-Diels-Alder reactions between polymers containing electron-deficient dithioesters and cyclopentadienyl end-capped polymers.\cite{35,36}

![Diagram](image_url)

**Figure 1.8:** Strategies for combining RAFT polymerization and click chemistry using clickable monomers, clickable chain transfer agents, and post-polymerization modification.\cite{34}

Another approach for combining RAFT polymerization and click chemistry is the post-polymerization modification in which a latent functionality is transformed into a
clickable group. The most common way is to convert the thiocarbonyl thio moiety into a reactive thiol with a primary amine (aminolysis) or NaBH$_4$ (reduction). The resulting thiol can be used subsequently for thiol-ene reactions or thiol-thiol coupling. Alternatively, the post-polymerization method is used to transform functionalities in the polymer chain for click reactions. For instance, alkyne groups for CuAAC are usually protected with a trimethylsilyl (TMS) or triisopropylsilyl (TIPS) group to avoid side reactions like cross-linking during the RAFT polymerization. These groups need to be removed with HCl (for TMS) or triethylamine·3HF (for TIPS) before conducting CuAAC.

1.3 Hydrogels

A hydrogel is a cross-linked, water-insoluble polymer, which absorbs and retains high quantities of water. There are many ways to divide hydrogels into classes. According to the cross-linking mechanism, hydrogels can be classified into two categories: (i) physical and (ii) chemical hydrogels. Chemically cross-linked hydrogels are formed by covalent bonds resulting in a permanent structure, while physically cross-linked hydrogels are generated by noncovalent attractive forces providing reversible linkages.

Chemical Hydrogels

Because chemical hydrogels are formed by covalent bonds, network formation can be achieved by (i) radical polymerization, (ii) chemical reaction of complementary groups (e.g. addition or condensation reactions, click chemistry, and many additional examples), (iii) irradiation, and (iv) enzyme-catalyzed reaction.

For polymerizations in which cross-linker and monomer react together in a radical process, common cross-linking agents are ethylene glycol diacrylate and $N,N'$-methylenebisacrylamide (BIS). A widely used initiator system is peroxydisulfate in combination with $N,N,N',N'$-tetramethylethane-1,2-diamine (TMEDA), which rapidly forms hydrogels with water-soluble vinyl monomers under ambient conditions. When the radical polymerization is conducted in organic solvents, a typical radical starter is azobisisobutyronitrile (AIBN). For photopatterning in microfluidics, a variety of different photoinitiators are available as well.

Cross-linking can be also achieved by functional groups (e.g. -OH, -COOH, -NH$_2$) fixed to the polymer backbone. For instance, glutaraldehyde (GTA) is a typical cross-linking agent for hydroxy and amine groups of water-soluble polymers. Moreover, Bigi et al. reported that 1 wt-% GTA is sufficient to obtain a gelatin film with a degree of cross-linking close to 100%. Hubbell and colleagues prepared cross-linked hydrogels
through a Michael-type addition reaction. Accordingly, PEG-dithiol rapidly forms cross-linked hydrogels able to encapsulate protein drugs without covalent modification. Beside addition reactions, click reactions between azide and alkyne groups can be employed for the synthesis of hydrogels. Lamanna and colleagues functionalized polysaccharide derivate with azide and alkyne groups, respectively. Aqueous solutions of both polymers with catalytic amounts of Cu(I) resulted in fast gel formation.

Because cross-linking by irradiation does not need additional additives (e.g. cross-linker, catalyst, among others), it is a popular technique for the preparation of hydrogels to be used in drug delivery. However, this technique requires that the cargo is encapsulated after the gel preparation (radical formation during the irradiation). Stringer and Peppas noted that PEG hydrogels can be prepared by gamma irradiation from the aqueous solution of high molecular weight PEG. More examples are poly(acrylic acid) (PAA), polyvinylpyrrolidone, and poly(vinyl alcohol) hydrogels, which can be obtained by electron beam or gamma irradiation from their respective polymers as well.

Another way to cross-link hydrogels is to utilize enzymes. This bioinspired approach is particularly interesting for the delivery of bioactive substances for tissue repair and reconstruction. Transglutaminase (TGase) is a $\text{Ca}^{2+}$-dependent enzyme that catalyzes the formation of isopeptide bonds. Hu and Messersmith employed TGase for the rapid formation of hydrogels composed of PEG-peptide conjugates. Fast gelation within minutes has been reported, which is particularly desired for medical applications. Horseradish peroxidase (HRP) is another useful enzyme to prepare hydrogels. The following reaction is catalyzed: $\text{ROOR'} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{ROH} + \text{R'}\text{OH}$. Lee et al. reported rapid network formation by HRP for branched 3,4-dihydroxyphenylalanine (DOPA) modified PEGs with two or more DOPA end-groups.

### Physical Hydrogels

Network formation of physical hydrogels can be achieved by (i) hydrophobic interactions, (ii) protein interactions, (iii) hydrogen bonds, and (iv) ionic interactions. An interesting example for a physical hydrogel by ionic interactions is the alginate hydrogel. Alginate is an unbranched glycuronan based on mannuronic acid and gluconic acid. Here, reversible gelation is achieved with calcium ions, which form intermolecular ionic bonds with the gluconic acid blocks. Interestingly, when a chelating agent for the calcium ions is added, the alginate hydrogel can be dissolved. Another example for ionic interactions is dextran, which forms degradable hydrogels with potassium ions. This is particularly surprising because dextran exhibits no ionic binding sites for positively charged ions. Watanabe et al.
revealed via NMR studies that six oxygen atoms of glucose units of three polymer chains form a cage in which the potassium ion perfectly fits.\(^7\)

Meijer and colleagues prepared physical hydrogels by hydrogen bonds.\(^7\) Poly(ethylene glycol) (PEG) based copolymers having ureidopyrimidinone units form supramolecular hydrogels with high strength and resilience upon deformation. More examples of physical hydrogels by hydrogen bonds are complexes formed by PEG with poly(methacrylic acid) (PMAA) or PAA.\(^7\) In these cases, hydrogen bonds between the ether oxygen of PEG and the carboxylic groups of PAA/PMAA are formed at low pH values. Interestingly, these complexes are stable in water at low pH values (e.g. \( \leq 5.7 \)), while the complexes dissolve in ethanol.\(^7\)

Miyata and colleagues presented additional cross-linking by protein interactions.\(^7\) In this example, rabbit immunoglobulin G (Rabbit IgG) as the antigen and goat anti-Rabbit IgG as the antibody were fixed to two individual polymer backbones. Antigen–antibody semi-interpenetrating polymer networks (semi-IPN) were prepared, which swell or shrink depending on the intra-chain antigen–antibody binding. Kopeček and colleagues reported another instance of hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains.\(^7\) Here, a metal complex is formed between the \( N-\left(N',N'-\text{dicarboxymethylaminopropyl}\right)\text{methacrylamide} \) (DAMA) in poly(\( N-\left(2\text{-hydroxypropyl}\right)\text{methacrylamide-co-DAMA} \)) and \( \text{Ni}^{2+} \), which is attached to the terminal histidine (His) residues of the His-tagged DAMA.

**Gelation Process in Free Radical Polymerization and Controlled Radical Polymerization**

There are two different gelation processes of hydrogels depending on the type of polymerization used: (i) free radical polymerization or (ii) controlled radical polymerization. Figure 1.9 shows the difference of the gelation process between FRP and CRP. Hydrogels prepared via FRP are characterized by slow initiation, fast chain propagation, and exclusive radical termination reactions.\(^7\) Note that due to slow initiation step compared to the fast propagation, primary radicals are constantly formed throughout FRP. At the beginning of the gelation process by FRP, primary radicals generated from the initiator lead to a dilute polymer solution with an extremely low polymer concentration. For this reason, vinyl groups of the cross-linker react mainly via intramolecular cyclization resulting in the formation of highly cross-linked nanogels. Naturally, more and more dense nanogels are formed as the gelation process proceeds. At higher monomer conversions, these nanogels are connected by newly generated radicals to nanogel clusters and form finally a gel with a highly heterogeneous structure.\(^7\)
Hydrogels can be obtained via CRP, such as ATRP or RAFT. It is worth noting that hydrogels obtained via CRP are classified as graft copolymer gels. Unlike free radical polymerization, rapid formation of primary radicals from the initiator leads to a steady number of propagating polymer chains at the beginning of the polymerization. Consequently, the number of propagating chains during the polymerization is more or less equal to the amount of added initiator. The gelation process via CRP starts with a dilute polymer solution as in FRP. At higher conversions, branched copolymers are obtained, which form a gel as the reaction proceeds. Importantly, due to the slower reaction kinetic of CRP, the propagating polymer chains relax and diffuse throughout the polymerization. For this reason, monomer and cross-linker can be homogeneously incorporated into the gel assuming equivalent reactivity of each vinyl species. A major difference of hydrogels prepared via CRP compared to FRP is the moment of gelation (i.e. gel point). When similar feed ratios are employed, the gel point of hydrogels prepared by CRP is delayed, while gelation of hydrogels via FRP occurs already at low conversion. This phenomenon is caused by the fast initiation step in CRP.

Stimuli-Responsive Hydrogels

Many applications of hydrogels in drug delivery, catalysis, microfluidics, among others, require controlled water uptake and release. By employing responsive monomers
so-called “smart” hydrogels are received, which absorb and release water as a result of changes in their environment. A wide variety of stimuli inducing volume phase transition is available for hydrogels, including temperature, pH value, ions, enzymes, magnetic forces, light, electrical, and shear forces (Figure 1.10).[84]

<table>
<thead>
<tr>
<th>Temperature-responsive</th>
<th>Light-responsive</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiPAAm</td>
<td>DEAAm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH-responsive</th>
<th>Glucose-responsive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anionic</td>
<td>Cationic</td>
</tr>
<tr>
<td>AA</td>
<td>VBA</td>
</tr>
</tbody>
</table>

**Figure 1.10:** Selection of pH-, temperature-, light-, and glucose-responsive monomers (NiPAAm = N-isopropylacrylamide, DEAAm = N,N-diethylacrylamide, VME = vinyl methyl ether, VCI = vinylcaprolactam, AA = acrylic acid, VBA = 4-vinylbenzoic acid, DEAEMA = N,N-diethyl aminoethyl methacrylate, 4VP = 4-vinylpyridine, VI = vinyl imidazole, SPAA = spirobenzopyran-functionalized AA, AAPBA = 3-(acrylamido)-phenylboronic acid).

pH-responsive hydrogels, also known as polyelectrolyte hydrogels, are composed of ionizable pendant groups, which are ionized at a specific pH value resulting in charges along the polymer backbone. These charges lead to a high osmotic pressure associated with swelling of the polyelectrolyte hydrogel. According to the ionizable pendant groups and the resulting swelling properties, polyelectrolyte hydrogels can be classified into anionic and cationic hydrogels. Anionic hydrogels are ionized and swollen when the concentration of H$_3$O$^+$ is greater than the acid dissociation constant $K_a$ (pH $>$ p$K_a$), whereas cationic hydrogels are ionized and thus swollen at concentrations of H$_3$O$^+$ less than $K_a$ (pH $>$ p$K_a$). Note that 50% of pendant groups are ionized in solution at pH = p$K_a$. Consequently, monomers with p$K_a$ values between 3 and 10 are suitable for polyelectrolyte...
1.3 Hydrogels

hydrogels. Figure 1.10 shows the chemical structures of common pH-responsive monomers for polyelectrolyte hydrogels, including acrylic acid (AA), 4-vinylbenzoic acid (VBA), $N,N$-diethylaminoethyl methacrylate (DEAEMA), and 4-vinylpyridine (4VP).\cite{84}

Guan and Zhang reported that boronic acid containing hydrogels are promising candidates for self-regulated insulin delivery.\cite{85} Figure 1.11 shows the glucose response of boronic acid based on the equilibrium among phenylboronic acid 14, negatively charged phenylboronic acid 15, and D-glucofuranose 1,2-borate complex 16. It is important to note that the complex between uncharged (alkylamido)phenyl boronic acid 14 and glucose in water is unstable due to its high tendency to undergo hydrolysis.\cite{86} On the contrary, negatively charged phenylboronic acid 15 complexes glucose to give a stable D-glucofuranose 1,2-borate 16 and water. In a series of studies, Kataoka and colleagues synthesized glucose-responsive hydrogels containing BIS, $N$-isopropylacrylamide (NiPAAm) and 3-(acrylamido)-phenylboronic acid (AAPBA).\cite{86-88} These boronic acid containing hydrogels exhibit an extraordinary change in the swelling degree triggered by glucose suitable for on-off regulation of insulin.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure11.png}
\caption{(left) The equilibrium among phenylboronic acid 14, negatively charged phenylboronic acid 15, and D-glucofuranose 1,2-borate complex 16. (right) The isomerization equilibrium between protonated merocyanine 17 and spirobenzopyran 18.}
\end{figure}

Light-responsive hydrogels are interesting materials for many applications in microfluidics because light can be radiated by lasers or through photomasks in high-resolution allowing to trigger materials down to 1 micron.\cite{80,89} In order to receive light-responsive hydrogels, chromophores such as azobenzene and spirobenzopyran are mainly used.\cite{90,91} Figure 1.11 displays the isomerization equilibrium between merocyanine 17 and spirobenzopyran 18. Under acidic conditions, the merocyanine 17 is protonated and totally hydrophilic. On the contrary, a ring-closed hydrophobic spirobenzopyran 18 is formed when it is irradiated with visible or ultraviolet light.
Temperature-responsive hydrogels have been extensively studied in the last decades. The temperature response is caused by a delicate balance of hydrophilic and hydrophobic interactions between polymer chains and aqueous solution. Figure 1.10 shows the chemical structure of common temperature-responsive monomers, including vinyl methyl ether (VME), N,N-diethylacrylamide (DEAAm), vinylcaprolactam (VCl), and NiPAAm.[84] Net-PNiPAAm is probably the most investigated temperature-responsive hydrogel, which exhibits a volume phase transition temperature (VPTT) at ~ 32 °C in water.[92–95] Figure 1.12 displays the swelling properties of net-PNiPAAm hydrogels below and above the VPTT. At temperatures below the VPTT, hydrogen bonds between water and hydrophilic segments of PNiPAAm dominate and result in good solubility of the polymer in water. Consequently, net-PNiPAAm is swollen at temperatures below the VPTT. When the temperature increases, the hydrogen bonds become weaker, whereas hydrophobic interactions between isopropyl units of PNiPAAm become stronger. At temperatures above the VPTT, these hydrophobic interactions dominate and lead to inter-polymer chain association accompanied by shrinking of the net-PNiPAAm hydrogel.[80] The temperature phase transition of net-PNiPAAm is reversible and can be adjusted by copolymerization of NiPAAm. Several groups have reported that hydrophilic comonomers increase the VPTT, while hydrophobic comonomers decrease the VPTT.[80,96] However, note that NiPAAm sequence length significantly affects the temperature transition and long NiPAAm segments are needed to retain the thermoresponsive behavior of PNiPAAm.[97] Typically, the comonomer content should not exceed 5 mol-% in respect to NiPAAm.[93,98]

Figure 1.12: Swelling properties of net-PNiPAAm hydrogels below (left) and above (right) the VPTT.
Multi-Responsive Hydrogels

Multi-responsive hydrogels possessing a temperature and pH response are highly interesting for many applications in microfluidics because swelling and shrinking can be either achieved externally via heating and cooling or internally via a pH change of the swelling agent. Because temperature-responsive NiPAAm and pH-responsive AA are used in this work to prepare multi-responsive hydrogels for flow control in microfluidics, the following section is directed to literature reports, which combine both monomers in one hydrogel. Figure 1.13 shows different types of hydrogel architectures combining two different species of monomers, including copolymer gels, graft copolymer gels, semi-interpenetrating polymer networks (semi-IPN), and interpenetrating polymer networks (IPN).

<table>
<thead>
<tr>
<th>Copolymer gel</th>
<th>Graft copolymer gel</th>
<th>Semi-interpenetrating polymer network</th>
<th>Interpenetrating polymer network</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Copolymer gel" /></td>
<td><img src="image2.png" alt="Graft copolymer gel" /></td>
<td><img src="image3.png" alt="Semi-IPN" /></td>
<td><img src="image4.png" alt="IPN" /></td>
</tr>
</tbody>
</table>

**Figure 1.13:** Types of hydrogel architectures combining two different species of monomers (monomer A = orange, monomer B = blue): copolymer gel, graft copolymer gel, semi-interpenetrating polymer network, and interpenetrating polymer network.

In theory, a copolymerization is the simplest way to obtain a temperature- and pH-responsive hydrogel composed of NiPAAm and AA. Cho and colleagues investigated the effect of AA concentration on the VPTT in net-P(NiPAAm-co-AA) hydrogels. Temperature-induced phase transition behavior of PNiPAAm retains exclusively in net-P(NiPAAm-co-AA) hydrogels containing 10 mol-% AA. Unfortunately, the pH-induced shrinking and swelling in net-P(NiPAAm-co-AA) hydrogels containing 10 mol-% AA is poor pronounced and higher AA concentration leads to a loss of the temperature sensitivity in net-P(NiPAAm-co-AA). Several studies have confirmed this result. Consequently, random copolymer gels composed of NiPAAm and AA are unsuitable for obtaining hydrogels with pronounced temperature and pH response.

Numerous studies have reported on IPNs composed of PNiPAAm and PAA. Unlike copolymer gels, these IPNs exhibit pronounced pH and temperature response because each network retains its individual properties. However, it is important to note that photopatterned hydrogel particles are required for many applications in microfluidics in particular for this work. Unfortunately, IPNs via soft lithography are extremely difficult
to fabricate and have not been reported in the literature. For this reason, IPNs composed of PNiPAAm and PAA are not useful for this work.

An alternative approach is the semi-IPN, which is based on a cross-linked PNiPAAm network penetrate by a linear or branched PAA polymer. Like IPNs, both polymers retain their individual properties in semi-IPNs resulting in a pH- and temperature-responsive hydrogel. Interestingly, the VPTT of semi-IPNs composed of a PNiPAAm network and hydrophilic PAA shifts to lower values due to additional coulomb ion–dipole interactions. A disadvantage of semi-IPNs is the potential leakage of the penetrating polymer, which is particularly likely as well as undesired in microfluidics.

**Graft Copolymer Gels**

Graft copolymer gels are a useful alternative to overcome the disadvantages of copolymer gels (loss of the thermoresponsive behavior due to the comonomer), IPNs (difficult fabrication of structured particles via soft lithography), or semi-IPNs (leakage of the penetrating polymer). Okano and colleagues first reported of net-PNiPAAm-g-PNiPAAm hydrogels with rapid deswelling to temperature changes in 1995. Although the number of reports about graft copolymer gels is limited, several studies have reported improved swelling kinetics of graft copolymer gels with a PNiPAAm backbone.

![Figure 1.14: Synthesis of grafted net-PNiPAAm-g-PDMAEMA hydrogels via free radical polymerization.](image)

Wang *et al.* reported temperature- and pH-responsive net-PNiPAAm-g-poly(N-(2-(dimethylamino)ethyl)-methacrylamide) (PDMAEMA) hydrogels via FRP (Figure 1.14).
Because both responses were equally pronounced in net-PNiPAAm-g-PDMAEMA hydrogels, which is particularly interesting for microfluidics, the synthesis of the hydrogel will be discussed in the following section. Note that a grafting-through method is used, i.e. a macromonomer method, to receive grafted net-PNiPAAm-g-PDMAEMA hydrogels. In a first step, a homopolymer of DMAEMA with a terminal COOH group was prepared via radical polymerization using AIBN and 3-mercaptopropionic acid as the initiator and the CTA, respectively. Then, PDMAEMA was functionalized with hydroxyethyl methacrylate to the final macromonomer ($M_n = 13000$ g/mol, $M_w/M_n = 1.7$) by a Steglich esterification employing 4-dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC). It should be mentioned that the methacrylate unit is connected through two ester linkages with PDMAEMA, which is not well tailored for pH-responsive hydrogels due to the high susceptibility of ester bonds to hydrolysis.\cite{108} Notwithstanding, grafted hydrogels were subsequently prepared using NiPAAm, PDMAEMA, BIS, ammonium persulfate (APS) and TMEDA. Importantly, a low grafting density of 0.22 mol-% PDMAEMA resulted in an adequate pH response, while the temperature response retained with a sharp VPTT at 34 °C.

Figure 1.15: Grafted net-PNiPAAm-g-PAA hydrogels prepared by RAFT polymerization.\cite{49}

Beside the grafting-through method using macromonomers, graft copolymer gels can be obtained by CRP.\cite{79} Yu and Zheng reported net-PNiPAAm-g-PAA hydrogels prepared via RAFT polymerization (Figure 1.15).\cite{49} Because this is the only study addressing hydrogels composed of a PNiPAAm backbone and PAA graft chains, it will be briefly described in this section. First, a PAA macroinitiator ($M_n = 11000$ g/mol) was prepared under standard RAFT conditions employing 3-benzylsulfanylthiocarbonylsulfanylpropionic acid as
the CTA and AIBN as the initiator. Subsequently, graft copolymer gels with PNiPAAm as the backbone and PAA as the graft chains were prepared via RAFT polymerization (48 h, 70 °C). Although hydrogels prepared via CRP have the advantage of readily accessible graft copolymer gels, it is important to note that this method suffers from extraordinarily long reaction times. This is particularly undesired in soft lithography processes, where long reaction times lead to the enlargement of the initial 3D structures (i.e. blurring). For this reason, graft copolymer gels prepared via CRP are unsuitable for this work.

1.4 Hydrogels for Flow Control in Microfluidics

Microfluidics covers the science of manipulating small quantities of fluids using microscale devices with great potential in analysis, multiplexing, automation, and high-throughput screening.[43] Compared to conventional systems, microfluidics benefits from miniaturization resulting in shortened time of experiments, decreased sample as well as reagent consumption, and reduced overall costs.[109] Employing hydrogels as an active material in microfluidics is particularly attractive because they work self-sufficiently and are independent of external hardware. The gel autonomously converts an environmental alteration into mechanical work. Therefore, the gel works as a sensor and an actuator simultaneously. Until now, hydrogels have been used as chemostats, micropumps, storage elements, and chemo-mechanical valves in microfluidics.[83,110–114]

The following is a list of key requirements to use hydrogels as active material, e.g. as a chemo-mechanical valve, for integrable microfluidic components: (i) significant volume change, (ii) sharp phase transition, (iii) reversible expansion and contraction, (iv) adequate long-term mechanical stability, (v) accelerated response time, and (vi) capable of undergoing photopolymerization.[43] Existing studies about hydrogels as a chemo-mechanical valve reported mainly of hydrogels responsive to only one stimulus, including temperature,[115] pH value,[51] and solvent.[44,45] Studies concerning multi-responsive hydrogels for flow control used materials with highly unequal volume changes towards different stimuli,[110,116,117] The following chapter will review methods using hydrogels for microfluidic lab-on-a-chip applications.

Chemo-Mechanical Valve

The first chemo-mechanical valve based on a hydrogel was reported in 1981.[118] Osada and colleagues presented a PMAA membrane, which water permeability was modified with PEG. When the membrane was treated with small amounts of PEG, the water permeation and flow rate increases. On the contrary, when the PEG-treated PMMA
membrane was rinsed with an alkali solution (pH 8), the membrane recovers the initial low water permeability due to the dissociation of the PMAA/PEG complex. Moreover, flow rate studies indicated that the PMAA membrane treated with PEG accelerates the protein separation of albumin and hemoglobin compared to an untreated PMAA membrane. However, the approach of a PMAA membrane is limited to small flow rates. Fréchet and colleagues demonstrated in 1997 that a porous monolith grafted with net-PNiPAAm is able to regulate a fluid flow.\cite{119} Accordingly, the net-PNiPAAm hydrogel can shrink and expand in a reversible way to open or close the pores of the monolith.

The first chemo-mechanical valve for flow control in the milliliter per minute range was reported by Arndt and colleagues in 2000.\cite{116} Figure 1.16 shows the schematic of the chemo-mechanical valve. It comprises an inlet, a valve chamber, an actuator chamber filled with hydrogel particles, a bypass, and an outlet. As hydrogel particles, net-PNiPAAm, net-P(NiPAAm-co-(N-(2-(dimethylamino)ethyl)acrylamide)), and net-P(NiPAAm-co-(3-acrylamidopropanoic acid)) were used. Flow rate studies indicated that actuator chamber filled with hydrogel particles can regulate the fluid flow. Overall, the response to temperature, pH value, and water-solvent mixtures was demonstrated.

**Figure 1.16:** Schematics of two chemo-mechanical valves based on hydrogels: (left) chemo-mechanical valve according to Arndt *et al.*\cite{116} and (right) microvalve according to Beebe *et al.*\cite{51} The blue arrows denote the direction of the fluid flow in the flow channel.

In 2000, Beebe and colleague presented an integrable microvalve based on hydrogels.\cite{51} The approach of this study was to fill a microchannel with a precursor solution and to irradiate this solution through a photomask. As reported, the precursor solution was consisting of AA and 2-hydroxyethyl methacrylate (HEMA) in a molar ratio of 1:4, ethylene glycol dimethacrylate as the cross-linker (1.4 wt-%) and Irgacure 651 as the photoinitiator (3 wt-%). The resulting hydrogel exhibited pH-induced phase transition associated with swelling upon pH raise between pH 4 and 7. As seen in Figure 1.16, a T-shaped channel
was fabricated in which three hydrogel particles are located at the entrance of the side branch. Depending on the state of the hydrogel, the fluid can flow either straight in the T-shaped channel or through the side branch as well as straight in the T-shaped channel. However, the study showed exclusively microscope images of the working principle without flow rate studies.

**Chemo-Fluidic Membrane Transistor**

Frank and colleagues extended the concept of doormat microvalves to a chemo-fluidic mem-
brane transistor (CFMT). This valve design is particularly interesting for applications where stimulus for the valve and fluid have to be separated because of contamination issues. Figure 1.17 shows a 3D cross-sectional schematic of a CFMT. The microvalve consists of a thin polydimethylsiloxane (PDMS) membrane (~ 30 μm) sandwiched between a control channel (lower channel) and a flow channel (upper channel) in a crossed-channel architecture. As the bottom layer, the CFMT is sealed with a cover glass. A standard soft lithography process was used to fabricate this normally-closed microvalve composed of PDMS. The flow channels are 200 μm wide and 100 μm high with a rectangular cross-section. Note that the flow channel contains a barrier (i.e. channel break, 200 μm wide), which closes the flow channel tightly without being covalently bonded to the thin membrane. Below the channel break, a hydrogel particle is incorporated in the control channel. The hydrogel particle is surrounded by posts (d = 150 μm) to permanently enclose it in the

![Diagram of a CFMT](image-url)

**Figure 1.17:** 3D cross-sectional schematic of a CFMT.\cite{44,45}
control channel. The used hydrogel particle was prepared via copolymerization of NiPAAm and sodium acrylate. Swelling and shrinking of the hydrogel was achieved by the fluid in the control channel using water (hydrogel expands) or a water-ethanol mixture (hydrogel contracts).

Figure 1.18 depicts the working principle of the CFMT. When a pressure is applied in the flow channel and the hydrogel is collapsed in the control channel, the membrane deflects downward allowing a fluid flow in the flow channel (i.e. the valve opens). In contrast, when a pressure is applied in the flow channel and the hydrogel is swollen, the membrane cannot deflect downward because the hydrogel particle puts pressure on the membrane from below and keeps the membrane nicely in place. Consequently, the valve remains tightly closed without a fluid flow in the flow channel.

![Figure 1.18: Closed and opened CFMT. The red arrows denote the direction of the fluid flow in the flow channel.](image)

One of the major goals in the field of microfluidics is to fabricate complex microfluidic circuits with several different components (e.g. valves, mixers, and pumps) on the same device. Because the CFMT provides flow control without bulky external hardware, basic microfluidic circuits such as basic logic gates (i.e. AND, OR, NOT) are readily available. Moreover, also more sophisticated circuits can be fabricated, including an RS flip-flop and a chemo-fluidic oscillator. Figure 1.19 shows various microfluidic circuits based on the concept of the CFMT. Note that the level of complexity increases from left to right. The simplest circuit, which performs a logic operation, is the NOT gate. The NOT gate consists of two parallel flow channels ($c_1$ and $c_0$) provided with different solutions. One is a water-ethanol mixture ($c_1 = 40$ vol-% ethanol), while the other is pure water ($c_0 = 0$ vol-% ethanol). A CFMT controls the fluid flow of the $c_1$ channel with the water-ethanol mixture.
Furthermore, the \( c_0 \) channel (pure water) exhibits a meander structure leading to a high fluidic resistance in this channel. Both control channels are joined at the downstream end.

![Flow channel](image1)

![Control channel](image2)

![CFMT](image3)

**Figure 1.19:** Chemo-fluidic toolbox for microfluidic circuits. The level of complexity increases from left to right. \( T = \) chemo-fluidic membrane transistor, \( A = \) solution for control channel, \( c_1 = \) flow channel with water-ethanol mixture, \( c_0 = \) flow channel with pure water.\cite{45}

The NOT gate works in the following way. When the control channel of the CFMT is provided with a solution in which the hydrogel swells, the \( c_1 \) channel is closed and the \( c_0 \) channel provides solely the output with pure water. On the other hand, when the control channel of the CFMT is provided with a solution in which the hydrogel collapses, the \( c_1 \) channel opens and contributes also to the output. Because the fluidic resistance of the \( c_0 \) channel is significantly higher than of the \( c_1 \) channel, the output of the NOT gate is supplied exclusively by the \( c_1 \) channel with the water-ethanol mixture.

### 1.5 Characterization Methods for Hydrogels

**Hydrogel Composition**

IR spectroscopy is one of the most important methods of vibrational spectroscopy and can be used to determine the hydrogel composition. When a molecule is exposed to infrared radiation (12800 to 10 cm\(^{-1}\)), the molecule absorbs radiation of a specific frequency which induces vibrational excitation of covalently bonded atoms and groups. The main advantages of IR spectroscopy are simple hardware, minimal sample consumption, simple sample preparation, and low time demands. Figure 1.20 shows a schematic of a generic FT-IR spectrometer.
Typically, a generic FT-IR spectrometer consists of an IR source, an interferometer, a sample compartment, a detector, and a computer. The source generates radiation in the infrared region from 12800 to 10 cm\(^{-1}\). Common IR sources are the Nernst glower, Globar, coils of chrome-nickel alloy, high-pressure mercury as well as tungsten lamps. After passing the interferometer, the infrared radiation goes through the sample and reaches the detector. Finally, the Fourier transform is carried out by a computer to receive the IR spectrum.

For quantitative analysis using IR spectroscopy, the Beer–Lambert law is valid, expressed as:

\[
A = \varepsilon \cdot c \cdot l, \tag{1.1}
\]

where \(A\) is the absorbance, \(\varepsilon\) is molar absorptivity, \(c\) is the concentration in solution and \(l\) is the thickness (path length). Accordingly, there is a linear relationship between absorbance and concentration of a sample, which allows the determination of the hydrogel composition via baseline method.\(^{121, 122}\)

In order to determine the concentration of a substance in an unknown sample, the height of a specific band of standard samples with known concentrations needs to be compared with the unknown sample. Figure 1.21 displays the baseline method to determine the band height of an absorbance band. As seen, the peak height is defined as the distance between band maximum and baseline. The baseline is derived from the two wings of the band.\(^{123}\)
Alternatively, when broad or asymmetric absorption bands need to be employed, the area under the band is used instead of the band height. A calibration curve is constructed by plotting the absorbance (i.e. band height) versus concentration, as shown in Figure 1.21.

**Figure 1.21:** (left) Baseline method for determining the band height of an absorbance band.\(^{[123]}\) (right) Calibration curve based on infrared absorbance versus concentration.

**Response Time**

An important aspect for a hydrogel in microfluidics is the swelling-deswelling kinetic. For an application as a chemo-mechanical valve, rapid uptake and release of the swelling agents is demanded to provide fast opening and closing function. Consequently, it is from high interest to determine the response time of a hydrogel to a stimulus, also known as the characteristic time of swelling. The kinetics of swelling of gels was first reported by Tanaka and Fillmore in 1979.\(^{[124]}\) According to Tanaka and Fillmore, the time-dependent change of radius \(r\) of a spherical hydrogel particle is defined as (neglecting shear modulus):

\[
\frac{r(t_{\infty}) - r(t)}{r(t_{\infty}) - r(t_0)} = \frac{\Delta r(t)}{\Delta r(t = 0)} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} n^{-2} \exp\left(-\frac{n^2 \cdot t}{\tau}\right),
\]

(1.2)

where \(\tau\) is the characteristic time of swelling. The characteristic time \(\tau\) is related to the cooperative diffusion coefficient \(D_{\text{coop}}\) as follows:

\[
D_{\text{coop}} = \frac{r(t_{\infty})^2}{\pi^2 \cdot \tau}.
\]

(1.3)

For a hydrogel network, the cooperative diffusion coefficient describes the collective or individual chain motions and is proportional to the square of the gel radius \(r\). However, the collective diffusion model by Tannaka and Fillmore is exclusively applicable to spherical hydrogel particles. Recently, Krause *et al.* reported that the collective diffusion model
extended by the volume specific surface can be used to determine the cooperative diffusion coefficient of hydrogel geometries such as worm- and disk-shaped particles at any aspect ratio.\[^{125}\] In the case of a cylinder-shaped hydrogel, the corrected cooperative diffusion coefficient $D_{coop}$ is related to the correction factor $h$ as follows:

$$corr. D_{coop} = \frac{D_{coop}}{h},$$

and

$$h = \frac{1 + 2AR}{3AR},$$

where $AR$ is the aspect ratio ($AR = \text{length/diameter}$) of the cylinder-shaped hydrogel. Figure 1.22 depicts the procedure for determining the corrected $D_{coop}$ in this work. An air dried hydrogel was conditioned for 24 h in pH 9 buffer solution at 50 °C. Air drying is particularly essential to avoid shrinking barrier effects associated with insufficient deswelling in the conditioning stage. Subsequently, the sample was transferred into a petri dish with pH 9 buffer solution at room temperature (r.t. = 22 °C) in which the hydrogel swelled until equilibrium was reached. In order to follow the swelling process, optical microscope images were recorded every 10 minutes. The characteristic time $\tau$ was obtained by $\tau = \frac{1}{k}$ from the slope $k$ of the curve for $t >> 0$ in the plot of the logarithm of $\frac{\Delta d(t)}{\Delta d(t_0)}$ versus time.

**Figure 1.22:** Procedure for determining the corrected $D_{coop}$. An air dried sample is conditioned for 24 h in a pH 9 buffer solution at 50 °C. The temperature stimulus is applied by transferring the gel into a pH 9 buffer solution at r.t. (r.t. = 22 °C) in which the gel swells until equilibrium is reached.

**Rheological Properties**

Rheological analysis via oscillation testing is the most common method to yield information about the mechanical properties of a hydrogel material. Figure 1.23 shows the schematic design of a rheometer with a parallel plate geometry.
A sample is placed between two plates and a motor applies a sinusoidal strain (e.g. angular displacement) by rotating the bottom plate. A transducer is connected to the upper plate and measures the resulting stress in the material. The phase angle $\delta$ describes the phase difference between the applied strain and the resulting stress. Three cases of the phase angle $\delta$ are possible:

- $\delta = 0^\circ$ for purely elastic materials (e.g. steel)
- $0^\circ < \delta < 90^\circ$ for viscoelastic materials (e.g. hydrogels)
- $\delta = 90^\circ$ for purely viscous materials (e.g. water)

Another important rheological parameter is the complex modulus $G^*$. The complex modulus $G^*$ corresponds to the overall resistance of the material to deformation and is defined as follows:

$$|G^*| = \frac{\sigma_A}{\varepsilon_A},$$  \hspace{1cm} (1.6)$$

where $\sigma_A$ is the stress amplitude and $\varepsilon_A$ is the strain amplitude (Figure 1.23). Furthermore, the complex modulus can be described by the storage modulus $G'$ and the loss modulus $G''$. The storage modulus $G'$ represents the ability of the material to store energy (elastic portion), whereas the loss modulus $G''$ corresponds to the ability of the material to dissipate energy (viscous portion):

$$G' = |G^*| \cdot \cos \delta,$$  \hspace{1cm} (1.7)
and

\[ G'' = |G^*| \cdot \sin \delta, \quad (1.8) \]

The loss factor \( \tan \delta \) is the ratio of \( G'' \) to \( G' \) and represents mechanical damping as well as internal friction.[126] This value allows conclusions to be drawn from the sample. The following cases can be considered:

- liquid character for \( G''/G' > 1 \)
- gel point at \( G''/G' = 1 \)
- gel character for \( G''/G' < 1 \)
2 Motivation and Aim

Microfluidics covers the science of manipulating small quantities of fluids using microscale devices with great potential in analysis, multiplexing, automation, and high-throughput screening. As outlined in the theoretical background, responsive hydrogels are well-suited for flow control in microfluidic systems because the fluid flow can be regulated without any additional power source associated with weight and cost reduction of microfluidic systems. Until now, existing studies mainly reported of hydrogels responsive to only one stimulus for flow control, including temperature, pH value, and solvent. Consequently, the approach of hydrogel for flow control should be extended to multi-responsive materials with equal volume change towards multiple stimuli. Moreover, it would be beneficial when the flow regulation could be performed internally as well as externally by combining temperature and pH stimulus in one material. Among the variety of temperature- and pH-responsive monomers, NiPAAm and AA are considered as ideal building blocks to achieve a hydrogel with pronounced stimuli response. There are different architectures for receiving a temperature- and pH-responsive hydrogel with NiPAAm and AA (e.g. copolymer gels, IPNs, semi-IPNs or graft copolymer gels). Each approach has its inherent benefits and disadvantages. As depicted, grafted hydrogels with a temperature-responsive backbone and pH-responsive graft chains are a promising approach overcoming drawbacks of copolymer gels (loss of the thermoresponsive behavior due to the comonomer), IPNs (difficult fabrication of structured particles via soft lithography), and semi-IPNs (leakage of the penetrating polymer). However, studies about multi-responsive grafted hydrogels for flow control in microfluidics are comparatively rare and further research is needed to emphasize their real potential.

In this consequence, the overall aim of this work is the synthesis of temperature- and pH-responsive grafted hydrogels based on NiPAAm and AA for flow control in microfluidics. The following is a list of key requirements for multi-responsive hydrogels as a chemomechanical valve: (i) independently addressable stimuli, (ii) significant volume change optimally with an equal response to all stimuli, (iii) sharp phase transition, (iv) reversible expansion and contraction, (v) adequate long-term mechanical stability, (vi) capable of undergoing photopolymerization, and (vii) accelerated response time.
2 Motivation and Aim

The aim of this work: A pH-responsive macromonomer should be prepared. Subsequently, grafted hydrogels composed of a PNiPAAm backbone and PAA graft chains will be synthesized. Key properties of the grafted hydrogels, such as swelling degree at specific environmental conditions, swelling and shrinking rate, mechanical strength, and photopolymerization, need to be examined. As proof of concept, grafted hydrogels should be used for flow control in a microfluidic device.

The aim will be accomplished by fulfilling the following objectives (comprised in Figure 2.1):

- For the synthesis of pH-responsive macromonomers, the chain transfer agent 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid will be functionalized at the radical leaving group R. The following functionalization will be tested: (i) allyl, (ii) unconjugated vinyl, (iii) azide, and (vi) alkyne. Then, pH-responsive macromonomers based on AA with different molecular weight will be synthesized via RAFT polymerization. When an azide or alkyne is used as the end-group, the obtained polymers will be functionalized via CuAAC with a corresponding counterpart containing a vinyl end-group. Importantly, the resulting polymers need to be investigated with respect to the degree of end-group functionalization. High end-group functionalization is essential (> 80 %) to ensure quantitative incorporation of the macromonomer into the hydrogel network.

- Subsequently, grafted hydrogel composed of a PNiPAAm backbone and PAA graft chains will be prepared. The grafting efficiency of the macromonomer into the grafted hydrogel need to be calculated. The influence of (i) the molecular weight of the macromonomer, (ii) the monomer type of the macromonomer, (iii) the grafting density, and (vi) the reaction conditions on the swelling properties of grafted hydrogels
will be studied as well. The swelling studies will focus on the volume change upon a stimuli and the volume phase transition. Furthermore, the reversibility of water uptake and release will be evaluated. Besides the swelling studies, grafted hydrogels will be investigated in terms of mechanical stability, the rate of the volume phase transition, and photopolymerization.

- As a proof of concept, grafted hydrogels will be used for flow control as a chemomechanical valve in microfluidics. Because an equal response to all stimuli is demanded in many microfluidic applications, the ideal grafting density will be determined using optical microscopy. A straightforward fluidic test station will be constructed in which the fluid flow is regulated by the expansion and contraction of the hydrogels within an actuator chamber. Furthermore, grafted hydrogels will be tested in a microfluidic chip in which hydrogel particle and flow channel are separated by a thin membrane.
Results and Discussion
3 Macromonomer Synthesis

3.1 Introduction

The aim of this study was to prepare multi-responsive grafted hydrogels for flow control in microfluidics as a chemo-mechanical valve. These grafted hydrogels should consist of temperature-responsive NiPAAm as the backbone, BIS as the cross-linker, and the pH-responsive macromonomer as the graft chain (Figure 3.1). Because NiPAAm and BIS are commercially available, only the pH-sensitive macromonomer needed to be synthesized. This chapter describes the synthesis of the pH-responsive macromonomer using different species of monomers and functional groups for the incorporation into the hydrogel network. In order to prepare the macromonomer, RAFT as the polymerization technique was selected. This controlled radical polymerization technique offers good functional group tolerance and a variety of polymerizable monomers under relatively mild reaction conditions.\[^6\] It is noteworthy that the reactivity of the CTA depends on its R and Z group. Therefore, the choice of the RAFT agent is directed to the selected class of monomers. In this work, acrylate and styrene monomers were chosen due to the high numbers of commercially available monomers sensitive to pH. As a suitable CTA for these monomers and with a carboxylic acid group for an end-group functionalization, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DTP) was employed. The approach towards the synthesis of the pH-responsive macromonomer with DTP based on two key steps: (i) attaching a functional group, which retains during RAFT polymerization, and (ii) conducting the RAFT polymerization to synthesize the pH-responsive macromonomer. Importantly, in order to provide long-term stability of the final grafted hydrogel, the functionalization of DTP with an amino group was aimed because amide bonds appear to be highly stable towards hydrolysis.\[^{108}\] Moreover, different functional groups of the macromonomer needed to be tested in terms of grafting efficiency into the hydrogel network. Other aspects were the molecular weight as well as the monomer type of the macromonomer. Because the number of reports on multi-responsive grafted hydrogels is limited, the ideal composition for an adequate temperature and pH response had to be found. Thus, macromonomers with different molecular weight and monomer types were prepared.
3 Macromonomer Synthesis

Figure 3.1: Components used in this work: NiPAAm as the temperature-responsive backbone of the hydrogel, BIS as the cross-linker, and the pH-responsive macromonomer as the graft chain. The approach to synthesize the macromonomer is (i) attaching a functional group, which retains during RAFT polymerization, and (ii) conducting the polymerization to prepare the pH-responsive macromonomer.

3.2 Allyl-Functionalization

As mentioned in the introduction, RAFT polymerization using DTP was chosen to prepare the pH-responsive macromonomer. Importantly, high end-group functionalization of the macromonomer (> 80 %) was demanded to provide maximum grafting efficiency within the hydrogel network. It is notable that the homopolymerization of acrylates or styrenes via RAFT polymerization using a vinyl-functionalized CTA can be viewed as a copolymerization of two vinyl monomers. Thus, a significant difference in the reactivity of the two vinyl groups is of crucial importance to achieve a well-defined vinyl-functionalized polymer. Because, theoretically, allyl groups are less reactive than acrylate groups, allyl end-group functionalization was selected as a suitable modification.\textsuperscript{[128]} Interestingly, the asymmetrical divinyl monomer, allyl methacrylate (AMA), exhibits both vinyl groups and is a useful reference point for this study. Several reports with different results are directed to the polymerization of AMA via CRP. For example, Bach \textit{et al.} prepared poly(allyl methacrylate) via surface-initiated RAFT polymerization.\textsuperscript{[129]} The study outlined that in the early polymerization stage of AMA via ATRP well-defined block copolymers are
formed. However, higher conversion of AMA was associated with cross-linking due to the side reaction of the allyl groups. Accordingly, selectivity between the two vinyl groups was insufficient to prepare high molecular allyl-functionalized block copolymers. On the contrary, Zhang et al. synthesized allyl-functionalized linear and star polymers with high molecular weight via RAFT polymerization. However, in this study, the employed allyl-functionalization (R−S−CH₂−CH=CH₂) exhibits lower reactivity compared to the allyl group of AMA (R−O−CH₂−CH=CH₂) due to the absence of the ester bond. 

Figure 3.2: (A) Preparation of two allyl-functionalized macromonomers using a two-step synthesis. Step 1 - Conversion of DTP to DTP-allyl amide or DTP-allyl ester using PyBOP and DIPEA as the coupling agents. Step 2 - Homopolymerization of AA employing DTP-allyl amide or DTP-allyl ester as the RAFT agents. (B) The structures of DCC, DMAP, PyBOP, and DIPEA.

Figure 3.2 shows the synthetic pathway to the allyl-functionalized PAA macromonomer. In order to functionalize the DTP raft agent with an allyl group, the carboxylic acid group was employed. However, a disadvantage of the used RAFT agent is that DTP rapidly undergoes thioamidation (aminolysis) with primary or secondary amines. The reaction is caused by the nucleophilic attack of the amino group on the trithiocarbonate (C=S unit) and leads to a thiol and subsequently to a dithiocarbamate. In fact, the functionalization of DTP with 2-propen-1-amine to 1-(allylamino)-2-methyl-1-oxopropan-2-yl dodecyl carbonotrithioate (DTP-allyl amide) in CH₂Cl₂ was attempted using a standard Steglich reaction with DCC and DMAP as the coupling agents (Figure 3.2B). In order to avoid aminolysis, the reaction was conducted with a slight excess of the coupling agents to equivalent amounts of DTP and 2-propen-1-amine (1.25 equivalents relative to DTP and 2-propen-1-amine). Although this Steglich approach was reported to provide high yields for the functionalization of dithioester (yields 80 %), the corresponding results
indicate a high degradation of DTP and low conversion (> 35 %) to the desired DTP-allyl amide (Table 1). A slightly improved result was achieved under argon atmosphere, but did not meet the expectation of good yields. Aiming an increase in conversion and to avoid the degradation of the RAFT agent by aminolysis, a more suitable coupling agent, benzotriazol-1-yl-oxy-tripyrrolidino-phosphonium hexafluorophosphate (PyBOP) with DIPEA, was employed (Figure 3.2B). PyBOP is a phosphonium type coupling reagent and is often used for the formation of amide bonds between amino acids and/or peptides.\textsuperscript{[139,140]} Indeed, high yields up to 90 % under an argon atmosphere were achieved using PyBOP and DIPEA (Table 3.1). \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy verified the chemical structure of DTP-allyl amide.

**Table 3.1:** Summary of coupling agents, conditions, and yields for the functionalization of DTP with 2-propen-1-amine in CH\textsubscript{2}Cl\textsubscript{2}.

<table>
<thead>
<tr>
<th>entry</th>
<th>coupling agents</th>
<th>conditions</th>
<th>yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DCC, DMAP</td>
<td>r.t., 2 d</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>DCC, DMAP</td>
<td>argon, r.t., 2 d</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>PyBOP, DIPEA</td>
<td>r.t., 2 d</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>PyBOP, DIPEA</td>
<td>argon, r.t., 2 d</td>
<td>90</td>
</tr>
</tbody>
</table>

Once DTP-allyl amide was prepared, allyl amide-functionalized poly(acrylic acid) (PAA-allyl amide) needed to be synthesized in the next step (Figure 3.2A). In order to prepare the PAA-allyl amide with high end-group functionalization, the polymerization kinetic was investigated in terms of monomer conversion and end-group functionalization. Accordingly, during the RAFT polymerization of AA using DTP-allyl amide in DMF at 70 °C and 4,4'-azobis(4-cyanopetanoyl) (ACP) as the radical initiator, \textsuperscript{1}H NMR spectroscopy was used to monitor the polymerization process periodically. Samples of 0.25 ml were taken at 0, 0.5, 1, 2, 3, 4 and 6 hours from the reaction mixture. The [M]:[CTA]:[I] molar ratio was at 1500:10:1 with an initial monomer concentration of 3 M. As seen on the used molar ratio, the reaction was conducted with a high excess of the monomer to avoid cross-linking of the allyl groups. Furthermore, the polymerization was performed under a strict degassing procedure using repeated freeze—pump—thaw cycles. Based on the kinetic plot ln[M\textsubscript{0}/M\textsubscript{n}] versus time shown in Figure 3.3, the rate of the reaction was observed to be first order, as depicted by the linear relationship between ln[M\textsubscript{0}/M\textsubscript{n}] and time. Remarkably, no inhibition period during the early stages of the polymerization was observed indicating
3.2 Allyl-Functionalization

fast re-initiation of the radical leaving group as well as fast fragmentation of intermediate RAFT radical. This result underlines that the chosen RAFT agent is highly suitable for the polymerization of AA. In order to determine the end-group functionalization, the resonance intensities of the methyl protons at 0.85 ppm were compared with the values of the vinyl protons at 5.75 ppm. Unfortunately, the resonance intensities of the vinyl protons decrease constantly during the polymerization emphasizing that the allyl group is polymerized under these reaction conditions. The final polymer PAA-allyl amide, obtained with 50 % monomer conversion after 6 h of polymerization, had a molecular weight of 5600 g/mol and 9 % end-group functionalization. However, this degree of end-group functionalization is insufficient for further use because it does not provide adequate vinyl bonds for the incorporation into the hydrogel network.

![Figure 3.3: End-group functionalization and ln\[M_0/M_n\] plotted as a function of the reaction time.](image)

Table 3.2 summarizes the polymerization data for the homopolymerization of AA. Three reaction parameters were varied to increase the end-group functionalization of allyl amide-functionalized PAA: (i) the solvent, (ii) the feed composition, and (iii) the bonding between allyl group and CTA. In order to avoid undesired cross-linking of the allyl end-group, toluene was employed for the following experiments as the reaction medium. Because toluene is a bad solvent for PAA, the formed allyl amide-functionalized PAA becomes insoluble during the polymerization and precipitates, which stops the polymerization in an early stage and the allyl-functionalization of the precipitated polymer is inaccessible for undesired cross-linking. However, the $^1$H NMR results revealed that this approach leads just to a slightly increase of the end-group functionalization up to 20 - 25 % (Entry 2-4 in Table 3.2). Furthermore, the feed composition was altered in terms of the
employed monomer concentration. With a higher excess of AA, the radical propagation with monomer units is more likely than with the allyl group. Unfortunately, $^1$H NMR spectroscopy showed no substantially improved end-group functionalization resulting from higher monomer concentration. Another aspect was the bonding of the allyl group and the CTA. Theoretically, allyl esters are less reactive than allyl amides due to the better resonance-stabilized radical. Allyl 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DTP-allyl ester) was prepared following the optimized procedure described for DTP-allyl amide (yield = 55%). The moderate yield of DTP-allyl ester compared to DTP-allyl amide may be attributed to the lower nucleophilicity of the hydroxyl group compared to the amine group. $^1$H and $^{13}$C NMR spectroscopy verified the chemical structure of DTP-allyl ester. As done before, end-group functionalization during the polymerization of PAA-allyl ester was investigated with $^1$H NMR spectroscopy. Two different feed compositions were employed (Entry 5/6 in Table 3.2). In fact, PAA-allyl ester polymers exhibited higher end-group functionalization compared to PAA-allyl amide (~ 55% vs ~ 25%), but this was still insufficient to provide adequate end-group functionalization for further usage as graft chains.

Table 3.2: Polymerization data and end-group functionalization (EGF) for the homopolymerization of AA using DTP-allyl amide and DTP-allyl ester as the RAFT agents. All polymerizations were conducted with ACP as the radical initiator.

<table>
<thead>
<tr>
<th>entry</th>
<th>CTA</th>
<th>feed ratio$^a$</th>
<th>solvent</th>
<th>EGF $^b$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DTP-allyl amide</td>
<td>1500:10:1</td>
<td>DMF</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>DTP-allyl amide</td>
<td>300:10:1</td>
<td>toluene</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>DTP-allyl amide</td>
<td>600:10:1</td>
<td>toluene</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>DTP-allyl amide</td>
<td>1200:10:1</td>
<td>toluene</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>DTP-allyl ester</td>
<td>300:10:1</td>
<td>toluene</td>
<td>54$^c$</td>
</tr>
<tr>
<td>6</td>
<td>DTP-allyl ester</td>
<td>600:10:1</td>
<td>toluene</td>
<td>55$^c$</td>
</tr>
</tbody>
</table>

$^a$ I = ACP, M = AA. $^b,c$ EGF = end-group functionalization, determined by $^1$H NMR spectroscopy comparing the signal of the terminal CH$_3$ (0.85 ppm) and $^b$: vinyl group (5.75 ppm) or $c$: vinyl group (5.25 ppm).

3.3 Unconjugated Vinyl-Functionalization

Because all attempts concerning an allyl-functionalization failed in terms of high end-group functionalization, an alternative strategy for the functionalization was developed.
Wooley et al. prepared well-defined polymers bearing alkene functionalities on the basis of Q- and e-values from Alfrey-Price theory.\textsuperscript{[128]} This approach of asymmetrical divinyl monomers is highly suitable for selective RAFT polymerization and was applied for the functionalization of DTP in this work (Figure 3.4). According to this strategy, the reactivity of the vinyl-functionalization was reduced by introducing an additional CH\textsubscript{2} group between DTP and vinyl-functionalization (unconjugated vinyl-functionalization R−O−CH\textsubscript{2}−CH\textsubscript{2}−CH=CH\textsubscript{2}). Note that the bonding for the functionalization was accomplished by an esterification because 3-buten-1-ol is cheaper and easier to obtain compared to but-3-en-1-amine. The synthesis of but-3-en-1-yl 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DTP-vinyl ester) was conducted analog to the optimized synthesis of DTP-allyl amide. The conversion was moderate with 65 %. \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy verified the chemical structure of DTP-vinyl ester.

\[ \text{DTP} \xrightarrow{\text{PyBOP, DIPEA}} \text{DTP-vinyl ester} \]

\[ \xrightarrow{\text{DMF, r.t. 2 d, Ar}} \]

\[ \xrightarrow{\text{ACP, DMF, 70 °C, Ar}} \text{PAA}_{\text{n-vinyl ester}} \]

\[ n=46,89,122 \]

Figure 3.4: Preparation of PAA\textsubscript{n-vinyl ester} using a two-step synthesis. Step 1 - Conversion of DTP to DTP-vinyl ester with 3-buten-1-ol employing PyBOP and DIPEA as the coupling agents. Step 2 - Homopolymerization of AA using DTP-vinyl ester as the RAFT agent.

RAFT polymerization in DMF at 70 °C was conducted for the synthesis of PAA\textsubscript{n-vinyl ester} with target molecular weights of 4000, 9800, and 18100 g/mol. Table 3.3 summarizes the polymerization data for the homopolymerization of AA using DTP-vinyl ester as the RAFT agent. \textsuperscript{1}H NMR analysis of entry 1 showed that the conversion reached 92 % (M\textsubscript{n,tho}= 4000 g/mol, M\textsubscript{n,NMR} = 3700 g/mol). For polymerizations targeting higher molecular weights, lower conversions of 69 % (M\textsubscript{n,tho} = 9800 g/mol, M\textsubscript{n,NMR} = 6800 g/mol) and 50 % (M\textsubscript{n,tho} = 18100 g/mol, M\textsubscript{n,NMR} = 9200 g/mol) were achieved due to the increased viscosity of the reaction medium at high conversions. The molar masses determined with SEC were in reasonable agreement although lower values were determined. SEC characterization of the PAA\textsubscript{n-vinyl ester} polymers revealed moderate molecular weight distributions (D = M\textsubscript{w}/M\textsubscript{n} ~ 2) with tailing at low molecular weight indicating that the polymerization was controlled. Several studies have reported high dispersity of PAA via RAFT polymerization.\textsuperscript{[141,142]} The hypothesis of these studies is that radical transfer to solvent at high conversion as well as at high [AA]/[CTA] ratios occurs, which is associated with less control over the polymerization.

In order to investigate the grafting efficiency of the prepared PAA\textsubscript{n-vinyl ester}, a redox polymerization of NiPAAm with PAA\textsubscript{n-vinyl ester} was conducted (Figure 3.5). Logically,
Table 3.3: Polymerization data for the homopolymerization of AA using DTP-vinyl ester as the RAFT agent. All polymerizations were conducted in DMF with ACP as the radical initiator.

<table>
<thead>
<tr>
<th>entry</th>
<th>feed ratio$^a$</th>
<th>$M_n$,$\text{theo}$$^b$</th>
<th>$M_n$,$\text{NMR}$$^c$</th>
<th>$M_n$,$\text{SEC}$$^d$</th>
<th>$\overline{D}$$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[M]:[CTA]:[I]</td>
<td>[g/mol]</td>
<td>[g/mol]</td>
<td>[g/mol]</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>[1000]:[20]:[1]</td>
<td>4000</td>
<td>3700</td>
<td>2000</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>[2600]:[20]:[1]</td>
<td>9800</td>
<td>6800</td>
<td>4900</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>[4900]:[20]:[1]</td>
<td>18100</td>
<td>9200</td>
<td>7200</td>
<td>2.1</td>
</tr>
</tbody>
</table>

$^a$ $M$ = AA, CTA = DTP-vinyl ester, I = ACP. $^b$ Calculated with $M_n$,$\text{theo} = [M]/[CTA] \times M_{\text{AA}} + M_{\text{DTP-vinyl ester}}$. $^c$ Determined by $^1$H NMR spectroscopy comparing the signal of the terminal CH$_3$ (0.85 ppm) and CH=C=O (2.0-2.4 ppm). $^d$ Determined by SEC in water using PAA calibration. $\overline{D} = M_w/M_n$.

the same reaction conditions were employed as for the planned hydrogel synthesis. The only difference was that the reaction was conducted without BIS as the cross-linker to avoid cross-linking accompanied by reduced solubility. Note that the trithiocarbonate of PAA$_n$-vinyl ester is still polymerizable and trithiocarbonates can undergo RAFT polymerization in water initiated by redox initiators.$^{[143-145]}$ Accordingly, three outcomes of the control experiment were possible: (i) a grafted PNiPAAm-$g$-PAA$_{46}$-vinyl ester block copolymer indicating a FRP of NiPAAm and PAA$_{46}$-vinyl ester, (ii) a PNiPAAm-$b$-PAA$_{46}$-vinyl ester block copolymer indicating a RAFT polymerization of NiPAAm with PAA$_{46}$-vinyl ester as the CTA, or (iii) a mixture of PNiPAAm and PAA$_{46}$-vinyl ester indicating a FRP of NiPAAm without PAA$_{46}$-vinyl ester.

Figure 3.5 shows the 2D-DOSY NMR spectrum of this experiment (log diffusion coefficient versus $^1$H NMR chemical shift). 2D-DOSY NMR spectroscopy is a useful characterization method for this experiment because it separates individual components in a mixture according to their diffusion coefficient.$^{[146]}$ It should be mentioned that the exact investigation of the diffusion coefficients was not the purpose of this study, the values must be considered relatively to each other. Two species with different diffusion coefficient representing PAA$_{46}$-vinyl ester (log D = -10.55 m$^2$/s) and PNiPAAm (log D = -11.15 m$^2$/s) are clearly visible. This leads to three important conclusions: (i) the chain transfer agent retains and does not undergo RAFT polymerization at conditions for the hydrogel synthesis, (ii) PAA-vinyl ester is too unreactive for the incorporation into the hydrogel network by redox initiation, and (iii) the functionalization of DTP with a unconjugated vinyl group is unsuitable for this study.
3.4 Acrylamide-Functionalization

Having failed to use allyl- and unconjugated vinyl groups as the end-group functionalization for the RAFT agent, an approach based on post-functionalization of the polymer via orthogonal ligation chemistry was developed. Because copper-catalyzed azide-alkyne cycloaddition in combination with RAFT is highly effective in terms of selectivity and reactivity, it was chosen for the preparation of the end-group modification in this study.\[15\] In order to successfully employ this approach, it requires that one of the two compatible click-functional groups is introduced into the RAFT agent, whereas the second group
is used for the corresponding counterpart. Normally, the counterpart should provide a vinyl or acrylate unit besides the click-functional group, which can be incorporated into the hydrogel network. Figure 3.6 displays the synthetic pathway for pH-responsive macromonomers by RAFT polymerization and CuAAC. As a useful reference study, Summerlin et al. reported on the functionalization of DTP with 3-azidopropan-1-ol for polymer-protein bioconjugates.\cite{147} However, because amide bonds were aimed for the functionalization, the amine analog 3-azidopropan-1-amine (20) was selected as the click-functional group. The required precursor 20 was subsequently prepared according to Pike.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3_6.png}
\caption{(top) Synthesis of the precursors 3-azido-1-propylamine (20) and N-(prop-2-yn-1-yl)acrylamide (23). (bottom) Preparation of PVBA\textsubscript{n}-AAm and P4VP\textsubscript{n}-AAm using a three-step synthesis. Step 1 - Conversion of DTP to DTP-N\textsubscript{3} with 3-azido-1-propylamine (20) using PyBOP and DIPEA as the coupling agents. Step 2 - Homopolymerization of various monomers (AA, 4VP, VBA) employing DTP-N\textsubscript{3} as the RAFT agent. The azide-functionalization was degraded when AA was used. Step 3 - Modification of the azide-functionalized polymers with N-(prop-2-yn-1-yl)acrylamide (23) via copper-catalyzed azide-alkyne cycloaddition.}
\end{figure}
3.4 Acrylamide-Functionalization

...and colleagues.\textsuperscript{[148]} \textsuperscript{1}H NMR analysis of component 20 outlines the requested patterns as found in the literature. With the prepared precursor 20, 1-((3-azidopropyl)amino)-2-methyl-1-oxopropan-2-yl dodecyl carbonotrithioate (DTP-N\textsubscript{3}) was prepared following the optimized procedure described for DTP-allyl amide (yield = 90 \%). \textsuperscript{1}H NMR spectroscopy confirmed the successful preparation of DTP-N\textsubscript{3} with the CH\textsubscript{2} signal at 3.1 ppm and the terminal CH\textsubscript{3} signal at 0.85 ppm (signal a and c in Figure 3.7A). Additional characterization by ATR-IR analysis showed a pronounced band at 2087 cm\textsuperscript{-1}, hence revealing the presence of N\textsubscript{3} stretching.

![Chemical shift and ATR-IR spectra](image)

\textbf{Figure 3.7:} \textsuperscript{1}H NMR (A) and ATR-IR (B) spectra of DTP-N\textsubscript{3} (bottom), PVBA\textsubscript{26}-N\textsubscript{3} (middle), and PVBA\textsubscript{26}-AAm (top).
of the azide group in DTP-N$_3$ (Figure 3.7B). In the next step, the corresponding counterpart for the click reaction was synthesized. Naturally, this group required an alkyne unit for a coupling reaction with the azide-functionalized DTP-N$_3$. The precursor $N$-(prop-2-yn-1-yl)acrylamide (23) was readily available from the reaction of prop-2-yn-1-amine (21) with acryloyl chloride (22). $^1$H and $^{13}$C NMR studies revealed the successful synthesis.

![Diagram of reaction schemes](image)

**Figure 3.8:** Control experiment to evaluate the degradation of the azide-functionalization of DTP-N$_3$ by AA and VBA. R group structures are postulated on findings of Perrier and colleagues.$^{[32]}$

After precursor 23 and DTP-N$_3$ were available, various PAA$_n$-N$_3$ homopolymers were synthesized using RAFT conditions analogous to the previously described approach for PAA$_n$-vinyl ester. Unfortunately, $^1$H NMR analysis failed to confirm the presence of the azide-functionalization due to overlapping of the propyl protons (CH$_2$−CH$_2$−CH$_2$−N$_3$) by the broad signals of the methylene groups of AA and water. Additionally, careful observation of the ATR-IR spectra of all three PAA$_n$-N$_3$ polymers showed the absence of the azide stretch at 2087 cm$^{-1}$ (Entry 1-3 in Table 3.4). Several groups reported that azide-functionalized polymers undergo 1,3-cycloaddition as a side reaction with electron-poor monomers at high temperatures and long reaction time.$^{[32,33]}$ For this reason, a control experiment was conducted with the objective to investigate if the azide group of DTP-N$_3$ is degraded by the electron-poor monomer AA at RAFT conditions. As a useful electron-rich monomer with a carboxylic acid group, VBA was investigated employing the same reaction conditions (Figure 3.8). In the experiment set-up, DTP-N$_3$ and the monomer of interest in excess (4 equivalents of AA or VBA in respect to DTP-N$_3$) were dissolved in DMF and heated at 80 °C for 20 h. In order to suppress RAFT polymerization and to observe exclusively the potential reaction between azide group and monomer, the
control experiment was conducted without a radical initiator. For evaluating the azide-functionalization of DTP-N$_3$, ATR-IR spectroscopy was chosen due to the pronounced azide stretch (Figure 3.9). ATR-IR analysis concerning the control experiment with AA showed that the characteristic stretch at 2087 cm$^{-1}$ disappeared after 20 h, which indicates that a 1,3-cycloaddition between azide group and monomer at RAFT conditions occurs. The product structures are postulated on findings of Perrier and colleagues.$^{[32]}$ On the other hand, when using VBA in the control experiment, both ATR-IR spectra showed no significant difference with the same intensity of the azide stretch. This illustrates that no reaction of the azide group and the VBA monomer proceeded. Consequently, electron-poor monomers like AA were left out and electron-rich monomers like VBA were used in all following studies.

![Figure 3.9: ATR-IR spectra of the control experiment shown in Figure 3.8. The azide stretch disappears in the control experiment with AA (bottom) and maintains in case of VBA (top).](image)

With the knowledge that only electron-rich monomers are suitable for the RAFT polymerization with DTP-N$_3$, homopolymers of VBA and 4VP were prepared under standard RAFT conditions employing DTP-N$_3$ in conjunction with ACP as the initiator in DMF at 80 °C. Table 3.4 summarizes the polymerization data for the homopolymerization of AA using DTP-N$_3$ as the RAFT agent. The determination of the azide-functionalization with $^1$H NMR failed due to signal overlapping of the propyl protons (−CH$_2$−CH$_2$−CH$_2$−N$_3$) with the methylene protons of the monomer units (−CH−CH$_2$−). Pleasingly, ATR-IR studies of PVBA$_n$-N$_3$ and P4VP$_n$-N$_3$ confirmed the presence of the azide group by the
characteristic stretch at 2087 cm\(^{-1}\) (PVBA\(_{26}\)-N\(_3\) in Figure 3.7B). In order to determine the molar masses of the prepared polymers (Entry 4 - 7), two different techniques, \(^1\)H NMR and SEC, were used. Concerning the \(^1\)H NMR study, end-group analysis comparing the terminal CH\(_3\) signal (0.85 ppm) and the phenyl-H signal (PVBA\(_n\)-N\(_3\): 7.4-7.9 ppm or P4VP\(_n\)-N\(_3\): 8.1 - 8.6 ppm) showed that the conversion reached ~ 50 - 70\%. These values were additionally confirmed by SEC providing slightly lower molar masses. Furthermore, SEC measurements revealed moderate dispersities with slight tailing at low molecular weight indicating that the polymerizations were controlled (\(\bar{D} = 1.4 - 1.8\)). MALDI-TOF MS analysis of entry 4 - 7 confirmed by the difference in m/z of the major peaks that the repeating units are VBA and 4VP, respectively (appendix Figure 9.1).

Table 3.4: Polymerization data for the homopolymerization of various monomers in DMF using DTP-N\(_3\) as the RAFT agent and ACP as the radical initiator.

<table>
<thead>
<tr>
<th>entry</th>
<th>monomer</th>
<th>feed ratio(^a)</th>
<th>([M]):([CTA]):([I]) [g/mol]</th>
<th>(M_{n,\text{theo}}^b) [g/mol]</th>
<th>(M_{n,\text{NMR}}^c) [g/mol]</th>
<th>(M_{n,\text{SEC}}^d) [g/mol]</th>
<th>(\bar{D})</th>
<th>azide band(^e)</th>
<th>EGF(^f)</th>
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<tbody>
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<td>-</td>
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<td>AA</td>
<td>2000:20:1</td>
<td>7600</td>
<td>6800(^e)</td>
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<td>-</td>
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<td>AA</td>
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<td>9200(^e)</td>
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<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>1000:20:1</td>
<td>7900</td>
<td>4400(^d)</td>
<td>3300(^f)</td>
<td>1.4(^f)</td>
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<td>90</td>
<td></td>
</tr>
<tr>
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<td>9000(^d)</td>
<td>8400(^f)</td>
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<tr>
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<td>2000:20:1</td>
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<td>5700(^g)</td>
<td>1.8(^g)</td>
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<td>68</td>
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</table>

\(^a\) I = ACP, CTA = DTP-N\(_3\). \(^b\) Calculated with \(M_{n,\text{theo}} = \frac{[M]}{[CTA]} \times M_{\text{monomer}} + M_{\text{DTP-N}_3}\). \(^c-e\) Determined by \(^1\)H NMR spectroscopy comparing the signal of the terminal CH\(_3\) (0.85 ppm) and c: CH–C=O (2.0-2.4 ppm), d: Phenyl-H (7.4-7.9 ppm), or e: Phenyl-H (8.1-8.6 ppm), respectively. \(^f\) Determined by SEC in f: THF using PS or g: DMAC using P2VP calibration. \(\bar{D} = M_w/M_n\). \(^h\) Detected by ATR-IR spectroscopy before click reaction.

EGF = end-group functionalization with an acrylamide unit was determined by \(^1\)H NMR spectroscopy comparing the signal of the terminal CH\(_3\) (0.85 ppm) and vinyl group (6.15 ppm).

In order to functionalize the PVBA\(_n\)-N\(_3\) and P4VP\(_n\)-N\(_3\) polymers with an acrylamide unit, the click reaction was performed in the next step. After the efficiency of the coupling was tested with a variety of Cu sources (CuBr, CuI, and CuSO\(_4\)/sodium ascorbate), ligands (DIPEA and PMEDTA), and solvents (DMF, DMF/H\(_2\)O, and THF), \(^1\)H NMR studies indicated that the catalyst CuSO\(_4\) in conjunction with sodium ascorbate in a water-DMF mixture gives the best results in terms of end-group functionalization (63 -
3.5 Styrene-Functionalization

Click chemistry was proven to be suitable as an end-group functionalization. However, the previously presented approach is limited to electron-rich monomers due to the degradation of the azide group by electron-poor monomers during the polymerization. Furthermore, the prepared PVBA\textsubscript{n}-AAm and P4VP\textsubscript{n}-AAm polymers are insoluble in water, which required the hydrogel synthesis in an organic solvent. Thus, an alternative approach was developed based on the functionalization of the RAFT agent with an alkyne group. Naturally, the corresponding counterpart should have a compatible azide group for the “click” reaction as well as a polymerizable group for the incorporation into the hydrogel network.

Figure 3.10 shows the synthetic pathway to the PAA\textsubscript{n}-styrene macromonomer employing RAFT and click chemistry. First, the RAFT agent DTP was functionalized in near quantitative conversion with propargylamine to dodecyl (2-methyl-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl) carbonotritioate (DTP-alkyne) using PyBOP and DIPEA as the coupling agents (yield 87 %). The high yield additionally highlights no side reaction of the CTA (no aminolysis). \textsuperscript{1}H and \textsuperscript{13}C NMR analysis confirmed the structure of DTP-alkyne. Furthermore, Raman spectroscopy showed strong absorbance bands at 1069 and 2124 cm\textsuperscript{-1} for the C=S and the C≡C stretch, respectively (Figure 3.11A). The counterpart for the click reaction 1-(azidomethyl)-4-vinylbenzene was readily synthesized according to O’Shea and colleagues. \textsuperscript{1}H NMR analysis outlined the requested patterns as found in the literature.\textsuperscript{[149]}

Three AA homopolymers were synthesized employing the functionalized DTP-alkyne as the CTA at 70 °C in DMF under an argon atmosphere with an initial monomer

90 %). Exemplarily, in the \textsuperscript{1}H NMR spectrum of PVBA\textsubscript{26}-N\textsubscript{3} (entry 4), the appearance of the proton signals at 5.58 and 6.11 ppm (signal i), attributed to CH\textsubscript{2} of the acrylamide unit, revealed the effective conjugation by formation of the triazole ring upon click reaction (Figure 3.7A). Careful observation of the ATR-IR spectrum showed the complete disappearance of the azide stretch at 2087 cm\textsuperscript{-1} (Figure 3.7B). Note that the reaction yields for polymers with a molar mass of 4400 g/mol were close to completion (~ 90 % for entry 4 and 6), while moderate reaction yields for polymers with higher molar masses were achieved (~ 65 % for entry 5 and 7). This may be attributable to the worse solubility of entry 5 and 7 in the water-DMF mixture used for the click reaction. As a result of the incomplete functionalization, these samples were not used in the following study. It should be mentioned that the prepared polymers PVBA\textsubscript{26}-AAm and P4VP\textsubscript{38}-AAm are exclusively soluble in organic solvent and exclude the hydrogel synthesis in water.
Figure 3.10: Preparation of PAA$_n$-styrene using a three-step synthesis. Step 1 - Conversion of DTP to DTP-alkyne with propargylamine using PyBOP and DIPEA as the coupling agents. Step 2 - Homopolymerization of AA in DMF employing DTP-alkyne as the RAFT agent. Step 3 - Modification of the alkyne-functionalized polymers with 1-(azidomethyl)-4-vinylbenzene via CuAAC.

Concentration of 2.95 M in a septa-sealed vial. As done before, the RAFT agent DTP-alkyne was used in high excess compared to the initiator ACP ([DTP-alkyne]:[ACP] = 20:1) to provide high control over the polymerization. Table 3.5 summarizes the polymerization data for the homopolymerization of AA using DTP-alkyne as the RAFT agent. For all samples, conversions above 80% were achieved indicating optimized conditions for the polymerization. SEC characterization of entry 1 and 2 revealed good molecular weight distributions (dispersities 1.5 and 1.6). In contrast, entry 3 shows a moderate dispersity of ~2. This may be attributable to the higher [AA]:[DTP-alkyne] feed ratio resulting in a less controlled polymerization.$^{[141,142]}$ Although $^1$H NMR analysis failed to confirm the presence of the alkyne-functionalization due to the overlapping with the $-\text{CH}-\text{CH}_2-$ signals, careful observation of the Raman spectra revealed the terminal alkyne group of all three samples with the C≡C stretch at 2124 cm$^{-1}$ (Figure 3.11A). The attempt to additionally prove the synthesis of PAA$_n$-alkyne using MALDI-TOF MS analysis was unsuccessful.

Finally, the end-group modification of the PAA$_n$-alkyne polymers with 1-(azidomethyl)-4-vinylbenzene was conducted under optimized click conditions employing CuSO$_4$ in conjunction with sodium ascorbate in a water-DMF mixture. Figure 3.11B shows the $^1$H
Figure 3.11: (A) Raman spectra of DTP-alkyne (bottom), PAA$_{53}$-alkyne (middle) and PAA$_{53}$-styrene (top). (B) $^1$H NMR spectrum of PAA$_{53}$-styrene in D$_2$O. Note that the absence of the COOH signal was due to the addition of NaOD.

NMR spectrum and peak assignments for entry 2. The end-group functionalization was determined with the integration ratios of the terminal CH$_3$ signal (signal m, 0.85 ppm) and the proton of the triazole ring (signal a, 7.92 ppm). Remarkably, for all three polymers, integration ratios close to 1 were detected indicating quantitative conversion close to completion. Note that the absence of the COOH signal in the $^1$H spectrum was due to the addition of NaOD to improve the solubility of PAA$_{53}$-styrene. Raman analysis
additionally showed evidence of the successful conjugation by the absence of the C≡C stretch at 2124 cm\(^{-1}\) (Figure 3.11A).

Table 3.5: Polymerization data for the homopolymerization of AA in DMF using DTP-alkyne as the RAFT agent.

<table>
<thead>
<tr>
<th>entry</th>
<th>feed ratio(^a)</th>
<th>(M_{n,\text{theo}})(^b)</th>
<th>(M_{n,NMR})(^c)</th>
<th>(M_{n,SEC})(^d)</th>
<th>(\frac{C≡C}{\text{band}})(^e)</th>
<th>EGF(^f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>5000</td>
<td>4200</td>
<td>3700</td>
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</tr>
<tr>
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<td>1900:20:1</td>
<td>7200</td>
<td>5700</td>
<td>4800</td>
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</tr>
</tbody>
</table>

\(\text{a } M = \text{AA, CTA = DTP-alkyne, I = ACP.}\)\(^b\) Calculated with \(M_{n,\text{theo}} = \frac{[M]}{[\text{CTA}]} \times M_{\text{AA}} + M_{\text{DTP-alkyne}}\). \(^c\) Determined by \(^1\)H NMR spectroscopy comparing the signal of the terminal CH\(_3\) (0.85 ppm) and CH–C=O (2.0-2.4 ppm). \(^d\) Determined by SEC in water using PAA calibration. \(\frac{D}{M_n} = M_W/M_n\). \(^e\) Detected by Raman spectroscopy before click reaction. \(^f\) EGF = end-group functionalization with a styrene unit was determined by \(^1\)H NMR spectroscopy comparing the signal of the terminal CH\(_3\) (0.85 ppm) and triazole ring (7.92 ppm).

3.6 Chapter Summary

This chapter was directed to the synthesis of various macromonomers for multi-responsive grafted hydrogels. For this aim, a suitable functionalization for the RAFT agent had to be found, which retains during RAFT polymerization, whereas it can be covalently incorporated into the hydrogel network by radical polymerization. As seen in Figure 3.12, four functionalizations for the macromonomer were investigated, including allyl, unconjugated vinyl, acrylamide, and styrene. As a suitable chain transfer agent for the RAFT polymerization, DTP was chosen due to the terminal carboxylic acid group, which was easily functionalized by amidation or esterification.

Starting with an allyl-functionalization, DTP was conjugated with 2-propen-1-amine to DTP-allyl amide using two different coupling agents. High conversions of the RAFT agent without aminolysis by the primary amine were exclusively observed with PyBOP and DIPEA. However, \(^1\)H NMR studies of the RAFT polymerization employing AA as the monomer showed that the allyl group was polymerized under RAFT conditions. An alternative RAFT agent DTP-allyl ester was investigated but led to the same result of
insufficient end-group functionalization. For this reason, the allyl-functionalization was unsuitable as an end-group for the RAFT agent.

**Figure 3.12:** Four different end-group functionalizations for the synthesis of macromonomers were investigated: (i) an allyl-functionalization, (ii) a unconjugated vinyl-functionalization, (iii) an acrylamide-functionalization, and (iv) a styrene functionalization.

A unconjugated vinyl-functionalization was examined. The functionalized RAFT agent DTP-vinyl ester was synthesized following the optimized approach with PyBOP and DIPEA. $^1$H NMR analysis of the RAFT polymerization confirmed the appearance of the vinyl peaks without degradation. Unfortunately, careful observation of the 2D-DOSY spectrum of the control experiment revealed that the unconjugated vinyl-functionalization macromonomer was not grafted into the PNiPAAm polymer. Consequently, the unconjugated vinyl-functionalization was not employed in this study.
Having failed to use allyl and unconjugated vinyl groups as an end-group functionalization, two alternative approaches based on click chemistry were developed. In the first approach, the RAFT agent was functionalized with an azide group and subsequently polymerized employing various monomer types. ATR-IR analysis showed that the azide-functionalization was degraded by electron-poor monomers (e.g. AA), while the azide group maintained with electron-rich monomers (e.g. VBA and 4VP). The prepared PVBAₙ-N₃ and P4VPₙ-N₃ macromonomers were functionalized with an acrylamide group using click chemistry. CuSO₄ in conjunction with sodium ascorbate in water-DMF mixture gave the best results in terms of end-group functionalization. Unfortunately, the hydrogel synthesis with the two macromonomers PVBAₙ-AAm or P4VPₙ-AAm was limited on organic solvents because both macromonomers are insoluble in water.

In order to overcome this limitation, DTP was modified with an alkyne group to DTP-alkyne. Three PAAₙ-alkyne polymers with different molar masses were successfully prepared using DTP-alkyne as the RAFT agent. The click reaction to introduce a styrene functionalization was close to completion following the same conditions with CuSO₄ and sodium ascorbate in DMF/H₂O.
4 Grafted Hydrogels Prepared in Organic Solvent

4.1 Introduction

This chapter is directed to the synthesis of multi-responsive grafted hydrogels employing the prepared macromonomers PVBA$_{26}$-AAm and P4VP$_{38}$-AAm. In order to establish multi-responsive hydrogels as a chemo-mechanical valve, the following key characteristics had to be fulfilled: (i) independently addressable stimuli, (ii) significant volume change optimally with an equal response to all stimuli, (iii) sharp phase transition, (iv) reversible expansion and contraction, (v) adequate long-term mechanical stability, (vi) capable of undergoing photopolymerization, and (vii) accelerated response time. Because the number of reports about grafted multi-responsive hydrogels for flow control in microfluidics is limited, it was crucial to identify the ideal feed ratio of NiPAAm and macromonomer. Naturally, the amount of incorporated macromonomer had to be large enough to introduce pH response, while it had to be small enough to retain the temperature response of PNiPAAm. Thus, a series of grafted hydrogels with different amounts of macromonomers were prepared and evaluated in terms of the swelling degree applying temperature and pH response. Because it is known that net-PNiPAAm hydrogels exhibit a response to salt as well as water-solvent mixtures, these both sensitivities needed to be investigated for the grafted hydrogels as well. Once suitable sensitivities were identified, the reversibility of those had to be tested by multiple cycling between swollen and collapsed state. Another important aspect was the mechanical stability of grafted hydrogel, which was tested in this work by rheology analysis. Note that results of chapter 4 are published in *RSC Advances*, 28-Mar-2016.

4.2 Synthesis and Composition

In order to obtain the ideal hydrogel composition for a suitable stimuli response, a series of grafted hydrogels was synthesized using NiPAAm as the hydrogel backbone, the prepared macromonomer PVBA$_{26}$-AAm or P4VP$_{38}$-AAm as the graft chains, AIBN as the
radical starter, and BIS as the cross-linker. The precursor solutions contained 1.25 mmol NiPAAm, 18.6 μmol BIS, and 12.5 μmol AIBN in 1 ml solvent. Because PVBA_{26-}AAm and P4VP_{38-}AAm are insoluble in water, the organic solvent pyridine was chosen as the reaction medium (Figure 4.1A). Importantly, low grafting density of the macromonomers from 0.25 to 1 mol-% was selected to retain the temperature response of the PNiPAAm backbone. Theoretically, this results in an average number of 50 monomer units between cross-links assuming ideal network formation. As a useful reference point for the response studies, a pure net-PNiPAAm hydrogel was prepared (Entry 1 in Table 4.1). Note that all polymerizations were conducted under strict degassing conditions employing three repeated freeze-pump-thaw cycles.

In total, 7 different hydrogels were prepared, including a net-PNiPAAm and two grafted net-PNiPAAm-g-PVBA_{26-}AAm and net-PNiPAAm-g-P4VP_{38-}AAm hydrogels. (Figure 4.1A). Both grafted hydrogels revealed similar properties in terms of swelling-deswelling behavior and stimuli response towards temperature. However, because net-PNiPAAm-g-P4VP_{38-}AAm were mechanically unstable, especially for higher grafting densities, the results will be mainly discussed for grafted net-PNiPAAm-g-PVBA_{26-}AAm hydrogels. Additional swelling-deswelling experiments and response studies of net-PNiPAAm-g-P4VP_{38-}AAm are located in the appendix.

**Table 4.1:** Feed ratios for net-PNiPAAm (entry 1), net-PNiPAAm-g-PVBA_{26-}AAm (entry 2-4), and net-PNiPAAm-g-P4VP_{38-}AAm (entry 5-7). All polymerizations were conducted in pyridine.

<table>
<thead>
<tr>
<th>entry</th>
<th>Macromonomer</th>
<th>Macromonomer content [mol-%]</th>
<th>feed ratio</th>
<th>NiPAAm [mmol]</th>
<th>Macromonomer</th>
<th>BIS [μmol]</th>
<th>AIBN [μmol]</th>
</tr>
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<td>18.6</td>
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<td>1.25</td>
<td>1.25</td>
<td>12.5</td>
<td>18.6</td>
<td>12.5</td>
</tr>
<tr>
<td>5</td>
<td>P4VP_{38-}AAm</td>
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<td>1.25</td>
<td>1.25</td>
<td>3.13</td>
<td>18.6</td>
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</tr>
<tr>
<td>6</td>
<td>P4VP_{38-}AAm</td>
<td>0.5</td>
<td>1.25</td>
<td>1.25</td>
<td>6.25</td>
<td>18.6</td>
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<td>P4VP_{38-}AAm</td>
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<td>12.5</td>
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</table>

An important aspect is the hydrogel composition, which allows access to the grafting efficiency of the macromonomer. According to literature, determination of the hydrogel
composition by ATR-IR analysis using the baseline method gives good results in terms of accuracy.\cite{121,122} Logically, before recording an ATR-IR spectrum, all synthesized hydrogels were washed several times with water to remove impurities. Careful observation of the net-PNiPAAm and net-PNiPAAm-g-PVBA_{26}-AAm spectra show characteristic C=O stretching at 1700 (PVBA_{26}-AAm) and 1657 cm\(^{-1}\) (PNiPAAm). For net-PNiPAAm-g-PVBA_{26}-AAm, the intensity of the C=O stretching at 1700 cm\(^{-1}\) increases with PVBA_{26}-AAm content.

Figure 4.1: (A) Synthesis of net-PNiPAAm-g-PVBA_{26}-AAm (left), net-PNiPAAm (middle), and net-PNiPAAm-g-P4VP_{38}-AAm (right). BIS = N,N’-methylenebisacrylamide, AIBN = azobisisobutyronitrile. (B) ATR-IR spectra of net-PNiPAAm-g-PVBA_{26}-AAm with different grafting densities (color code in the left part of the figure corresponds to the composition of the curves in the right part).
In order to quantify the composition of the net-PNiPAAm-g-PVBA\textsubscript{26}-AAm hydrogels, a series of VBA/NiPAAm solutions varying in the molar composition from 0.05 to 0.21 was prepared (appendix Figure 9.2). Note that the intensity was normalized using the NiPAAm C=O stretch at 1657 cm\textsuperscript{-1}. Subsequently, a calibration curve showing the VBA/NiPAAm ratio as a function of ATR units at 1700 cm\textsuperscript{-1} was calculated by linear regression (appendix Figure 9.2). The determined hydrogel compositions are summarized in Table 4.2. There is a good agreement between the theoretical and determined hydrogel composition, which indicates quantitative incorporation of PVBA\textsubscript{26}-AAm into the hydrogel network, thus a good accessibility of the acrylamide end-group of the PVBA\textsubscript{26}-AAm macromonomer during the polymerization is given (grafting efficiency 72 to 99\%).

### Table 4.2: Ratio of NiPAAm/VBA, PVBA\textsubscript{26}-AAm content, and grafting efficiency determined via ATR-IR measurements and the baseline method.\textsuperscript{[121,122]}

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<th>Determined ratio of NiPAAm/VBA</th>
<th>Theoretical PVBA\textsubscript{26}-AAm content\textsuperscript{a}</th>
<th>Determined PVBA\textsubscript{26}-AAm content\textsuperscript{a}</th>
<th>Grafting efficiency\textsuperscript{b}</th>
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</tr>
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<td>88/12</td>
<td>89/11</td>
<td>0.5</td>
<td>0.49</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>79/21</td>
<td>82/18</td>
<td>1</td>
<td>0.87</td>
<td>87</td>
</tr>
</tbody>
</table>

\textsuperscript{a} In respect to NiPAAm. \textsuperscript{b} Calculated with grafting efficiency = determined PVBA\textsubscript{26}-AAm content / theoretical PVBA\textsubscript{26}-AAm content.

### 4.3 Response Behavior

There are two techniques to determine the swelling degree of a hydrogel sample: volumetric and gravimetric. Because the prepared hydrogel samples exhibited a highly heterogeneous disk shape, the swelling degree was exclusively calculated using the gravimetric approach. For this, a disk-shaped hydrogel sample of a thickness of \textasciitilde 5 mm was equilibrated for 1 d in the solution of interest. Subsequently, the equilibrated sample was taken out of the solution, the surface liquid was removed with a moistened filter paper, and the sample was weighed. The swelling degree $Q_{m,i}$ for the hydrogels was defined as follows:

$$Q_{m,i} = \frac{W_s}{W_d} - 1,$$

(4.1)
where $W_s$ is the weight of the swollen hydrogel and $W_d$ is the weight of the dried gel. Overall, the equilibrium swelling properties were studied at various pH values in buffer solution (2 - 10 at r.t. and 50 °C), temperatures (20 - 50 °C in pH 3 and pH 9 buffer solution), water-solvent mixtures (0 - 100 % with acetone, methanol, ethanol, and 1-propanol), and salt concentrations (0 - 1 M with NaI, NaSO$_4$, NaCl, MgCl$_2$, and AlCl$_3$). Importantly, in order to obtain a quantitative estimation of the stimuli response, the ratio of the swelling degree between swollen and collapsed state was determined by:

$$\text{ratio } Q_{m,i} = \frac{Q_{\text{swollen}}}{Q_{\text{collapsed}}},$$

where $Q_{\text{swollen}}$ is the swelling degree in the swollen state and $Q_{\text{collapsed}}$ is the swelling degree in the collapsed state. In compliance with literature reports, a stimuli ratio $Q_{m,i}$ from 3 to 5 is adequate to provide a sufficient volume change for an opening and closing function as a chemomechanical valve (volume change of about 66 to 80 % for a disk-shaped hydrogel).\textsuperscript{[110,114]} Notably, swollen and collapsed state were defined as states with the highest and lowest swelling degree and are highlighted by gray bars in the diagrams.

### pH Response

In order to realize a pH-responsive behavior in the PNiPAAm hydrogel, the PVBA$_{26}$-AAm macromonomer was incorporated as a graft chain into the hydrogel backbone. Because the temperature response of PNiPAAm should retain with a sharp transition and high stimuli response, low grafting density between 0.25 and 1 mol-% of the PVBA$_{26}$-AAm macromonomer was used. Figure 4.2 displays the equilibrium degree of swelling $Q_{m,pH}$ plotted as a function of the pH value and the determined ratio $Q_{m,pH}$ for net-PNiPAAm-g-PVBA$_{26}$-AAm samples with different grafting densities. The swelling degree was measured in buffer solution ranging from pH 2 to pH 10. Note that the pH dependence was investigated at r.t. and at 50 °C. Thus, the PNiPAAm hydrogel backbone was either swollen (at r.t., $T < \text{VPTT of NiPAAm}$) or collapsed (at 50 °C, $T > \text{VPTT of NiPAAm}$). The pH response was quantified employing pH 3 and pH 9 as swollen and collapsed state.

As expected, the swelling degree of pure net-PNiPAAm remains constant with 40 at r.t. (net-PNiPAAm swollen) and 2 at 50 °C (net-PNiPAAm collapsed) over the investigated pH range. This non-existent pH response of both temperatures is additionally revealed by the ratio $Q_{m,pH}$ values of ~ 1. In contrast, the equilibrium swelling degree of all net-PNiPAAm-g-PVBA$_{26}$-AAm hydrogels shows a sharp transition between pH 6 and pH 8 independently on whether the PNiPAAm backbone is swollen at r.t. or collapsed at 50 °C. Beyond this transition, below pH 6 and above pH 8, the swelling degree is
Figure 4.2: (left) Equilibrium swelling degree $Q_{m,\text{pH}}$ for net-PNiPAAm-$g$-PVBA$_{26}$-AAm samples with different grafting densities at r.t. (bottom) and 50 °C (top) plotted as a function of pH value. (right) Determined pH response ratio $Q_{m,\text{pH}}$ for net-PNiPAAm-$g$-PVBA$_{26}$-AAm samples with different grafting densities. In order to achieve an adequate pH response, the grafting density of PVBA$_{26}$-AAm must be higher than 0.25 mol-% (color code in the right part of the figure corresponds to the composition of the curves in the left part). More or less constant. Importantly, all grafted net-PNiPAAm-$g$-PVBA$_{26}$-AAm hydrogels reveal a pronounced discontinuous transition, which is needed for the application as a chemo-mechanical valve. The obtained swelling curves with a transition between pH 6 and pH 8 are in good agreement with the $pK_a$ value 7.1 of pure PVBA. For net-PNiPAAm-$g$-PVBA$_{26}$-AAm hydrogels, the pH sensitivity is caused by the deprotonated carboxylic acid groups above the $pK_a$ of PVBA. The resulting repulsion between the ionized carboxylic acid groups expand the grafted hydrogels and the appearance of mobile counterions leads to an additional swelling due to their osmotic pressure. Logically, higher grafting density of PVBA$_{26}$-AAm results in higher pH response when ionized. It is noteworthy that the pH response of net-PNiPAAm-$g$-PVBA$_{26}$-AAm hydrogels at r.t. is generally lower than at 50 °C due to the high deswelling when the PNiPAAm backbone is collapsed. Overall,
4.3 Response Behavior

sufficient pH response for an adequate volume change (ratio $Q_{m,pH} > 3$) is achieved for both temperatures with a grafting density of 0.5 mol-% PVBA$_{26}$-AAm (Figure 4.2).

![Figure 4.3](image-url)

**Figure 4.3:** (left) Equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-$g$-P4VP$_{38}$-AAm samples with different grafting densities at r.t. (bottom) and 50 °C (top) plotted as a function of pH value. (right) Determined pH response ratio $Q_{m,pH}$ for net-PNiPAAm-$g$-P4VP$_{38}$-AAm samples with different grafting densities. In order to achieve an adequate pH response, the grafting density of PVBA$_{26}$-AAm must be higher than 0.25 mol-% (color code in the right part of the figure corresponds to the composition of the curves in the left part).

Figure 4.3 displays the equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-$g$-P4VP$_{38}$-AAm samples with different grafting densities at r.t. (bottom) and 50 °C (top) plotted as a function of pH value. The swelling curves of net-PNiPAAm-$g$-P4VP$_{38}$-AAm show a sharp transition between pH 4 and pH 6 with more or less stable $Q_{m,pH}$ values beyond the phase transition ($pK_a = 5.62$). At low pH, the graft chains based on P4VP$_{38}$-AAm are protonated, thus positively charged, resulting in water uptake of the grafted hydrogel. Increasing the macrononomer content results in higher swelling degrees in the swollen state under acidic conditions. At high pH, the 4VP groups of the macrononomer are uncharged and swelling of the gel samples is reduced. Interestingly, the swelling degrees of
net-PNiPAAm-g-P4VP\textsubscript{38}-AAm are in the same range of net-PNiPAAm-g-PVBA\textsubscript{26}-AAm resulting in similar ratio $Q_{m,pH}$ values. An adequate volume change (ratio $Q_{m,pH} > 3$) is achieved for both temperatures with a grafting density of 0.5 mol-% P4VP\textsubscript{38}-AAm.

**Temperature Response**

As a next step, the effect of the pH-responsive graft chains on the temperature response was examined. Desirably, the temperature dependency of the hydrogels should be unaffected by the graft chains and retained with a sharp transition and a high stimuli response. Because net-PNiPAAm-g-PVBA\textsubscript{26}-AAm and net-PNiPAAm-g-P4VP\textsubscript{38}-AAm showed a similar response towards temperature, but net-PNiPAAm-g-PVBA\textsubscript{26}-AAm revealed better mechanical properties, the results will be exclusively discussed for net-PNiPAAm-g-PVBA\textsubscript{26}-AAm (appendix Figure 9.3 for net-PNiPAAm-g-P4VP\textsubscript{38}-AAm). In order to investigate the effect of a swollen or collapsed state of the PVBA\textsubscript{26}-AAm graft chain, the temperature response was analyzed at a pH above (in pH 9, where PVBA\textsubscript{26}-AAm graft chains are unprotonated and swollen) and below the pK\textsubscript{a} of VBA (in pH 3, where PVBA\textsubscript{26}-AAm graft chains are protonated and collapsed). Note that the temperature of the buffer solutions was increased from 20 to 50 °C in steps of 5 °C. The temperature response ratio $Q_{m,T}$ was determined with 20 and 50 °C as swollen and collapsed state.

Figure 4.4 shows the equilibrium swelling degree plotted as a function of the temperature and the determined ratio $Q_{m,T}$ for net-PNiPAAm-g-PVBA\textsubscript{26}-AAm samples with different grafting densities. For pure net-PNiPAAm in pH 3 and pH 9 buffer solution, the equilibrium swelling degree decreases from ~ 45 to ~ 1 when the temperature increases from 20 to 35 °C. At temperatures above 35 °C, the swelling degree is consistently below 1. For both pH values, the temperature response is highly pronounced with ratio $Q_{m,T}$ values > 100. This temperature dependency is provoked by the coil-globular transition of PNiPAAm, a delicate balance between hydrogen bonds and hydrophobic interactions. At temperatures below VPTT, hydrogen bond interactions between water and isopropyl groups of the polymer dominate resulting in swelling of the net-PNiPAAm hydrogel. On the contrary, at temperatures close or above VPTT, hydrophobic interactions between the amide groups of PNiPAAm increase associated with phase separation and deswelling of net-PNiPAAm.

The swelling curves of grafted net-PNiPAAm-g-PVBA\textsubscript{26}-AAm hydrogels decrease substantially with increasing temperature. This behavior was independently on whether the experiment was conducted in pH 3 or pH 9 buffer solution. Accordingly, all grafted net-PNiPAAm-g-PVBA\textsubscript{26}-AAm hydrogels retain their temperature sensitivity. However, the temperature dependency of grafted hydrogels differs depending on the ionization of the PVBA\textsubscript{26}-AAm graft chains. In pH 3 buffer solution at temperatures below 35 °C, when
4.3 Response Behavior

Figure 4.4: (left) Equilibrium swelling degree $Q_{m,T}$ for net-PNiPAAm-g-PVBA$_{26}$-AAm samples with different grafting densities in pH 9 (bottom) and pH 3 buffer solution (top) plotted as a function of temperature. (right) Determined temperature response ratio $Q_{m,T}$ for net-PNiPAAm-g-PVBA$_{26}$-AAm samples with different grafting densities. The temperature sensitivity retains with a suitable response for all samples (color code in the right part of the figure corresponds to the composition of the curves in the left part).

the PVBA$_{26}$-AAm graft chains are protonated and hydrophobic, higher PVBA$_{26}$-AAm content leads to smaller equilibrium swelling degrees of grafted hydrogels compared to pure net-PNiPAAm. When the temperature increases above 35 °C, grafted hydrogels are totally collapsed like pure net-PNiPAAm with swelling degrees of ~ 1. Importantly, the determined ratio $Q_{m,T}$ (pH 3) values decrease with PVBA$_{26}$-AAm content from 104 (0 mol-% PVBA$_{26}$-AAm) to 45 (1 mol-% PVBA$_{26}$-AAm) but are considerably above the prerequisite of ratio $Q_{m,i} > 3$. On the contrary, when the experiment is conducted in pH 9 buffer solution and the PVBA$_{26}$-AAm graft chains are ionized and hydrophilic, higher PVBA$_{26}$-AAm content results in higher swelling compared to pure net-PNiPAAm. Remarkably, because the PVBA$_{26}$-AAm graft chains remain ionized in pH 9 buffer solution, total water release of grafted net-PNiPAAm-g-PVBA$_{26}$-AAm hydrogels is prevented even when the temperature increases above the VPTT of PNiPAAm. This behavior is more
pronounced for grafted hydrogels with higher PVBA26-AAm content. Consequently, the temperature response of grafted hydrogels at pH 3 is superior compared to pH 9, which is revealed by a high drop of the ratio $Q_{m,T}(pH \, 9)$ values from 112 (0 mol-% PVBA26-AAm) to 10 (1 mol-% PVBA26-AAm). Note that all samples fulfill the prerequisite of ratio $Q_{m,i}$ values > 3 without restrictions.

Figure 4.5: Determined VPTT for net-PNiPAAm-g-PVBA26-AAm samples with different grafting densities in distilled water by (left) DSC and (right) UV/Vis light transmittance using the cloud point method.\[58\]

In order to determine the volume phase transition temperature (VPTT), differential scanning calorimetry (DSC) and UV/Vis were used. Figure 4.5 displays the DSC thermographs of net-PNiPAAm-g-PVBA26-AAm hydrogels with different grafting densities. Notably, the VPTT was determined in distilled water using the temperature of the endothermic peak (onset point). The net-PNiPAAm hydrogel shows the typically VPTT of 32.4 °C, which is in good agreement with literature reports.\[50\] The VPTT of grafted net-PNiPAAm-g-PVBA26-AAm hydrogels slightly shifts to higher values from 33.0 to 33.2 to 33.3 °C. Figure 4.5 displays the determined VPTT values using the cloud point method.\[156\] These VPTTs are congruent with the values calculated with DSC. Furthermore, the turbidity experiments additionally imply a sharp transition of net-PNiPAAm-g-PVBA26-AAm containing 1 mol-% PVBA26-AAm similar to pure net-PNiPAAm. This strong, discontinuous transition is particularly beneficial from the application point of view because a high volume change upon a small environmental alteration is highly demanded.
Swelling and Shrinking

Aiming an application of grafted net-PNiPAAm-g-PVBA26-AAm hydrogels as a chemomechanical valve, the swelling-deswelling behavior of net-PNiPAAm-g-PVBA26-AAm over repeated cycles using pH and temperature response was investigated. Desirably, the equilibrium swelling degree of swollen and collapsed state for all stimuli should be constant to provide reproducible opening and closing function as a chemomechanical valve.

Figure 4.6: (left) Equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-g-PVBA26-AAm samples with different grafting densities at r.t. (bottom) and 50 °C (top) when the hydrogel was transferred from pH 9 to pH 3 buffer solution, and vice versa. (right) Equilibrium swelling degree $Q_{m,T}$ for net-PNiPAAm-g-PVBA26-AAm samples with different grafting densities in pH 9 (bottom) and pH 3 buffer solution (top) when the temperature of the swelling agent was alternated between r.t. and 50 °C.
Figure 4.6 displays the swelling degree for net-PNiPAAm-\textit{g}-PVBA_{26}-AAm samples with different grafting densities when the samples were transferred from pH 9 to pH 3 buffer solution, and \textit{vice versa} (4 cycles in total). Note that the experiment was conducted at r.t. with a swollen PNiPAAm backbone (bottom) and at 50 °C with a collapsed PNiPAAm backbone (top). As expected from the pH response studies, pure net-PNiPAAm shows no oscillating swelling degree upon repeated pH switches between pH 9 and pH 3 buffer solution independent of the temperature. In contrast, grafted hydrogels exhibit a reproducible swelling-deswelling at r.t. and at 50 °C. Unfortunately, all net-PNiPAAm-\textit{g}-PVBA_{26}-AAm hydrogels exhibit a lower pH response after the first cycle, followed by stabilized equilibrium swelling degrees over the remaining pH switches. This tendency increases with PVBA_{26}-AAm content and is unaffected whether the PNiPAAm backbone is swollen or collapsed. For net-PNiPAAm-\textit{g}-PVBA_{26}-AAm containing 1 mol-% PVBA_{26}-AAm, the swelling degree in the swollen state (pH 9) drops after 4 cycles from 171 to 131 at r.t. and from 31 to 18 at 50 °C, whereas net-PNiPAAm-\textit{g}-PVBA_{26}-AAm containing 0.25 mol-% PVBA_{26}-AAm reveals a drop from 67 to 62 at r.t. and from 9 to 7 at 50 °C. Because net-PNiPAAm-\textit{g}-P4VP_{38}-AAm hydrogels exhibit a similar phenomenon in pH 3 buffer solution when the P4VP_{38}-AAm graft chains are swollen (appendix Figure 9.4), this may be attributed to ionic hydrations of the buffer solution by the graft chains resulting in a decreased osmotic pressure.\textsuperscript{[157]} Accordingly, the pH response is just partially suitable due to the decreasing pH response after four swelling-deswelling cycles.

Figure 4.6 shows the swelling degree for net-PNiPAAm-\textit{g}-PVBA_{26}-AAm with different grafting densities when the temperature of the swelling agent was alternated between r.t. and 50 °C. As done for the pH cycle, the experiment was conducted for two cases: one was in pH 9 buffer solution when the PVBA_{26}-AAm graft chains are swollen (top), while the other one was in pH 3 buffer solution when the PVBA_{26}-AAm graft chains are collapsed (bottom). In pH 9 buffer solution, the swelling-deswelling of all samples is reproducible over 4 cycles without considerable change in the equilibrium swelling degrees. In pH 3 buffer solution, on the contrary, the net-PNiPAAm hydrogel shows a pronounced swelling-deswelling upon repeated temperature changes, whereas net-PNiPAAm-\textit{g}-PVBA_{26}-AAm hydrogels reveal a poor expansion and contraction behavior. It is assumed that this phenomenon is provoked by hydrogen bonding between the isopropyl groups of the PNiPAAm backbone and the carboxylic acid groups of the PVBA_{26}-AAm graft chains as well as between carboxylic acid groups of individual PVBA_{26}-AAm graft chains (Figure 4.7). Because grafted net-PNiPAAm-\textit{g}-P4VP_{38}-AAm hydrogels exhibit an irreproducible swelling-deswelling when totally collapsed as well (appendix Figure 9.4), it is likely that π-interactions have an additional contribution. In consequence of these interactions, diffusion barriers occur and
prevents the water uptake of the grafted hydrogels at these conditions. For this reason, the temperature response at pH 3 is unsuitable for an application in microfluidics.

![Possible π-interactions and H-bonded complexes](image)

**Figure 4.7:** Possible π-interactions and H-bonded complexes between the amide groups of the PNiPAAm backbone and the carboxylic acid groups of the PVBA<sub>26</sub>-Aam graft chains as well as between carboxylic acid groups of individual PVBA<sub>26</sub>-Aam graft chains in pH 3 buffer solution.\textsuperscript{[38]}

### Salt Response

Tominaga et al. reported that salts lower the VPTT of net-PNiPAAm because of dehydration with respect to hydrophobic interactions.\textsuperscript{[158]} On the other hand, Xu and colleagues showed that net-PVBA exhibits high salt resistivity compared to net-PAA due to stabilized hydrogen bonds as a result of ionic hydrations.\textsuperscript{[157]} Thus, it was particularly important to investigate net-PNiPAAm-g-PVBA<sub>26</sub>-Aam and to look at it in contrast to net-PNiPAAm and net-PVBA hydrogels. Because net-PNiPAAm-g-PVBA<sub>26</sub>-Aam containing 0.5 mol-% PVBA<sub>26</sub>-Aam has suitable pH and temperature response for the application as a chemomechanical valve (ratio Q<sub>m,i</sub> > 3), all salt experiments were conducted with this hydrogel. Naturally, pure net-PNiPAAm was chosen as reference hydrogel.

Figure 4.8 presents the equilibrium swelling degree plotted as a function of the salt concentration (NaI, Na<sub>2</sub>SO<sub>4</sub>, NaCl, MgCl<sub>2</sub>, and AlCl<sub>3</sub>). As the swollen and the collapsed state for this experiment, the salt concentrations 0 and 1 mol/L were chosen. For a pure net-PNiPAAm, the swelling degree remains constant when the salt concentration increases from 0 to 0.1 mol/L. Above a salt concentration of 0.1 mol/L, the swelling curves show a significant drop for Na<sub>2</sub>SO<sub>4</sub>, NaCl, MgCl<sub>2</sub>, and AlCl<sub>3</sub>. NaI reveals exclusively a stable swelling degree of ~ 40 over the investigated concentration range. These results are in good agreement with literature reports, indicating that the phase transition of PNiPAAm...
directly correlates with the hydration entropy of the anion.\textsuperscript{[159]} Consequently, all salts except NaI show a suitable salt response with ratio $Q_{m,salt}$ values greater than 3, whereas NaCl and MgCl\textsubscript{2} exhibit the highest values with ratio $Q_{m,salt}$ of $\sim 40$.

**Figure 4.8:** (left) Equilibrium swelling degree $Q_{m,salt}$ for net-PNiPAAm (top) and net-PNiPAAm-\textit{g}-PVBA\textsubscript{26}-AAM (bottom) plotted as a function of the salt concentration using Na\textsubscript{2}SO\textsubscript{4}, NaCl, MgCl\textsubscript{2}, AlCl\textsubscript{3}, and NaI (bottom). (right) Determined salt response ratio $Q_{m,salt}$ of net-PNiPAAm (top) and net-PNiPAAm-\textit{g}-PVBA\textsubscript{26}-AAM containing 0.5 mol-% PVBA\textsubscript{26}-AAM (bottom). A suitable salt response is achieved with Na\textsubscript{2}SO\textsubscript{4}, NaCl, MgCl\textsubscript{2}, and AlCl\textsubscript{3} (color code in the right part of the figure corresponds to the composition of the curves in the left part).

In contrast, net-PNiPAAm-\textit{g}-PVBA\textsubscript{26}-AAM containing 0.5 mol-% PVBA\textsubscript{26}-AAM exhibits a less pronounced salt response. Net-PNiPAAm-\textit{g}-PVBA\textsubscript{26}-AAM containing 0.5 mol-% PVBA\textsubscript{26}-AAM is totally swollen at a salt concentration of 0 mol/L. Note that the equilibrium swelling degree in pure water is higher compared to buffer solution due to the lower ionic strength of water ($Q_{m,salt(0 M)} = 250 > Q_{m,pH \text{r.t.}} = 150$). When the salt concentration increases to 1 mol/L, the swelling degree drops discontinuously for Na\textsubscript{2}SO\textsubscript{4}, NaCl, MgCl\textsubscript{2}, and AlCl\textsubscript{3} to $\sim 50$ while it rises for NaI to $\sim 290$. Due to the lower deswelling of net-PNiPAAm-\textit{g}-PVBA\textsubscript{26}-AAM at a salt concentration of 1 mol/L, the salt response of
the grafted hydrogel is worse than of pure net-PNiPAAm hydrogels but still complies with the criterion of ratio $Q_{m,\text{salt}}$ values > 3 (except NaI).

**Solvent Response**

PNiPAAm responses not only to temperature, it also exhibits a pronounced sensitivity to compositions of water-solvent mixtures. This so named cononsolvency describes the phenomenon that two good solvents for a polymer turn into a bad solvent when mixed together. Several studies have reported cononsolvency of PNiPAAm for different water-solvent mixtures, such as water-methanol, water-ethanol, and water-DMF. Similar behavior is observed for net-PNiPAAm, which undergoes a swelling-collapse-swelling

![Figure 4.9](image)

**Figure 4.9:** (left) Equilibrium swelling degree $Q_{m,\text{solvent}}$ for net-PNiPAAm (top) and net-PNiPAAm-$g$-PVBA$_{26}$-AAm containing 0.5 mol-% PVBA$_{26}$-AAm (bottom) plotted as a function of volume fraction of solvent in water. (right) Determined solvent response ratio $Q_{m,\text{solvent}}$ of net-PNiPAAm and net-PNiPAAm-$g$-PVBA$_{26}$-AAm containing 0.5 mol-% PVBA$_{26}$-AAm. The solvent response is suitable for microfluidics (color code in the right part of the figure corresponds to the composition of the curves in the left part).
transition. The cononsolvency was investigated for four different water-solvent mixtures, including three alcohols (methanol, ethanol, and 1-propanol) and one ketone (acetone). In order to determine the solvent response, 0 and 40 vol-% of solvent in water were selected as swollen and collapsed state.

Figure 4.9 displays the equilibrium swelling degrees for net-PNiPAAm and net-PNiPAAm-g-PVBA_{26}-AAm containing 0.5 mol-% PVBA_{26}-AAm plotted as a function of the volume fraction of solvent in water. Both hydrogels show similar swelling curves upon increased solvent content. In pure water, all samples are totally swollen with swelling degrees of ~ 45 for net-PNiPAAm and ~ 250 for net-PNiPAAm-g-PVBA_{26}-AAm. At about 10 to 20 vol-% solvent, they totally collapse and re-swell again at about 50 to 60 vol-% solvent. At higher solvent concentration, between 70 and 90 vol-% solvent, the grafted net-PNiPAAm-g-PVBA_{26}-AAm and pure net-PNiPAAm are partially swollen with lower swelling degrees compared to 0 vol-% solvent. Interestingly, net-PNiPAAm-g-PVBA_{26}-AAm containing 0.5 mol-% PVBA_{26}-AAm reveals considerably higher solvent response compared to pure net-PNiPAAm. In any case, net-PNiPAAm-g-PVBA_{26}-AAm fulfills the prerequisites of ratio $Q_{m,\text{solvent}}$ values $> 3$ for all investigated solvents.

### 4.4 Mechanical Stability

One of the major limitations of hydrogels in microfluidic systems is their poor mechanical properties. The mechanical properties of hydrogels are mainly affected by two parameters: (i) the cross-linking density and (ii) the swelling degree. Because the cross-linking density of all prepared hydrogels is more or less equally assuming ideally network formation, the swelling degree is the determining parameter for the mechanical stability. There have been many techniques employed to study the mechanical properties of hydrogels. The most widely used method is to determine the shear and Young’s modulus by rheological analysis.\[163-165\] In this study, the storage modulus of the prepared hydrogels was estimated because it reveals the ability of a material to return energy and depicts how elastic the material is. Naturally, adequate storage modulus is desired to provide suitable mechanical stability for an application in microfluidics. It should be mentioned that three measurements were conducted and averaged at an angular velocity $\omega = 1$ rad/s. In order to ensure totally swollen hydrogels, all samples were equilibrated for 24 h in pH 9 buffer solution. Note that the storage modulus of all samples was linear over the investigated range $\omega = 0.1 - 100$ rad/s.

Figure 4.10 displays the storage modulus at $\omega = 1$ rad/s plotted as a function of the PVBA_{26}-AAm content. Because the storage modulus is inversely proportional to the
swelling degree,\textsuperscript{[163,166]} pure net-PNiPAAm shows the highest recorded storage modulus (6500 ± 400 Pa), thus the greatest mechanical properties. For net-PNiPAAm-g-PVBA\textsubscript{26}-AAm containing 0.25 mol-% PVBA\textsubscript{26}-AAm, a lower value of 5600 ± 700 Pa was detected, indicating slightly worse mechanical stability compared to net-PNiPAAm without graft chains. Unfortunately, due to their poor mechanical properties, all samples of grafted net-PNiPAAm-g-PVBA\textsubscript{26}-AAm hydrogels with a PVBA\textsubscript{26}-AAm content above 0.25 mol-% were destroyed by the applied compression and shear forces during the rheological analysis. (Figure 4.10 left). Concerning an application as an active material, net-PNiPAAm-g-PVBA\textsubscript{26}-AAm with adequate pH response (PVBA\textsubscript{26}-AAm content ≥ 0.5 mol-%) is exclusively suitable for microfluidic systems without any mechanical stress.

4.5 Chapter Summary

A series of grafted net-PNiPAAm-g-PVBA\textsubscript{26}-AAm hydrogels with a PNiPAAm backbone and PVBA\textsubscript{26}-AAm graft chains were synthesized. Importantly, low PVBA\textsubscript{26}-AAm content between 0.25 and 1 mol-% was selected to introduce adequate pH response and to retain the temperature response of PNiPAAm. Equilibrium swelling studies indicated...
that a PVBA$_{26}$-AAm content of 0.5 mol-% is sufficient to achieve an adequate swelling response to temperature and pH value (ratio $Q_{m,i} > 3$). Remarkably, the temperature transition retained sharp and was mainly unaffected by the incorporated PVBA$_{26}$-AAm graft chains. The swelling analysis employing various salts showed that net-PNiPAAm-g-PVBA$_{26}$-AAm containing 0.5 mol-% PVBA$_{26}$-AAm exhibits a less pronounced salt response compared to pure net-PNiPAAm due to stabilized hydrogen-bonding by PVBA$_{26}$-AAm. Notwithstanding, the salt response still met the criterion of ratio $Q_{m,salt} > 3$ (except for NaI). In addition, the consololvency of net-PNiPAAm-g-PVBA$_{26}$-AAm (0.5 mol-% PVBA$_{26}$-AAm) was analyzed by equilibrium swelling experiments using 4 different solvents. Highly pronounced deswelling between 20 and 50 vol-% solvent was detected suitable for an application as a stimulus in microfluidics.

![Temperature and pH response](image)

**Figure 4.11:** (left) Net-PNiPAAm-g-PVBA$_{26}$-AAm containing 1 mol-% PVBA$_{26}$-AAm at different conditions: (pH 9, r.t.) hydrogel totally swollen; (pH 9, r.t. and pH 9, 50 °C) hydrogel partially collapsed; (pH 3, 50 °C) hydrogel completely collapsed.$^{[38]}$ (right) Optical images of net-PNiPAAm and net-PNiPAAm-g-PVBA$_{26}$-AAm (0.5 mol-% PVBA$_{26}$-AAm) samples before, during, and after a rheological analysis employing compression and shear forces. All samples of net-PNiPAAm-g-PVBA$_{26}$-AAm with a PVBA$_{26}$-AAm content above 0.25 mol-% were destroyed during the rheological analysis by the applied forces due to the poor mechanical properties.

The swelling-deswelling studies revealed that all net-PNiPAAm-g-PVBA$_{26}$-AAm hydrogels exhibit a lower pH response after the first cycle, which may be attributed to ionic hydrations of the buffer solution by the graft chains resulting in a decreased osmotic pressure. Interestingly, grafted net-PNiPAAm-g-PVBA$_{26}$-AAm hydrogels showed oscillatory swelling degrees in pH 9 buffer solution applying temperature stimulus, while poor swelling-deswelling behavior was detected in pH 3 buffer solution (Figure 4.11). This result was explained by a hypothesis about the formation of intrachain complexes due
to π-interactions and hydrogen bonds between amide groups and protonated carboxylic
groups as well as between carboxylic groups of single graft chains, which prevent the water
uptake of grafted net-PNiPAAm-g-PVBA$_{26}$-AAm hydrogels. Furthermore, the mechanical
properties of the prepared materials were evaluated by the storage modulus using rheo-
logical analysis. Unfortunately, all samples of grafted net-PNiPAAm-g-PVBA$_{26}$-AAm
hydrogels in particular with a PVBA$_{26}$-AAm content ≥ 0.5 mol-% revealed extremely
poor mechanical properties unsuitable for microfluidic systems with any mechanical stress.
Because of the highly limited mechanical stability of grafted net-PNiPAAm-g-PVBA$_{26}$-
AAm hydrogels, other key properties like response time and photopolymerization were not
further investigated.
5 Grafted Hydrogels Prepared in Water

5.1 Introduction

The following chapter is directed to the synthesis and characterization of grafted net-PNiPAAm-g-PAA_n-styrene hydrogels with PAA_n-styrene as the macromonomer. Because grafted net-PNiPAAm-g-PVBA_26-AAm hydrogels synthesized in organic solvent reveal tetra-sensitivity without reasonable mechanical stability in particular for higher grafting density, net-PNiPAAm-g-PAA_n-styrene was exclusively prepared in water. One of the primary aims was to identify the ideal hydrogel composition. A pH response had to be introduced, while the temperature-sensitivity needed to be retained. In this case, the response behavior of grafted hydrogels was tested in terms of the grafting density as well as the molecular weight of the graft chains. Naturally, solvent and salt response needed to be investigated regarding feasibility for further tasks as well. Note that still all key properties for an application as a chemo-mechanical valve had to be fulfilled: (i) independently addressable stimuli, (ii) significant volume change optimally with an equal response to all stimuli, (iii) sharp phase transition, (iv) reversible expansion and contraction, (v) adequate long-term mechanical stability, (vi) capable of undergoing photopolymerization, and (vii) accelerated response time. Most results of chapter 5 are submitted to ACS Applied Materials & Interfaces, 29-Mar-2016.

5.2 Synthesis and Composition

Net-PNiPAAm hydrogels and net-PNiPAAm-g-PAA_n-styrene hydrogels were synthesized from precursor solutions containing 1.25 mmol NiPAAm, 18.6 μmol BIS, 12.5 μmol sodium persulfate (SPS) in conjugation with 6.63 μmol TMEDA, and 3.13–12.5 μmol macromonomer in 1 ml water (Table 5.1). Because PAA_n-styrene is just slightly soluble in neutral water, the hydrogel synthesis was conducted in alkaline water at pH 10. Moreover, in order to avoid heterogeneous reaction conditions resulting in translucent samples with poor swelling and shrinking properties, the precursor solution was constantly cooled in a water bath at 10 °C. In total, 6 different hydrogels samples were prepared, which
Figure 5.1: Synthesis of \textit{net}-PNiPAAm, \textit{net}-PNiPAAm-\textit{g}-PAA\textsubscript{29}-styrene, \textit{net}-PNiPAAm-\textit{g}-PAA\textsubscript{53}-styrene, and \textit{net}-PNiPAAm-\textit{g}-PAA\textsubscript{75}-styrene via free radical polymerization. BIS = \textit{N},\textit{N}'-methylenebisacrylamide, SPS = sodium persulfate, TMEDA = \textit{N},\textit{N},\textit{N}',\textit{N}'-tetramethylethane-1,2-diamine.

varied in the grafting density as well as the molecular weight of the graft chains (Figure 5.1). Furthermore, a pure \textit{net}-PNiPAAm hydrogel without graft chains was prepared as a reference point for this study. The molar composition of each hydrogel was determined via IR spectroscopy and the baseline method as described in chapter 4.2 (Figure 9.2). Based on these results, the

<table>
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<th>entry</th>
<th>Macro-monomer</th>
<th>Macro-monomer content\textsuperscript{a}</th>
<th>NiPAAm [mmol]</th>
<th>Macromonomer [mol-%]</th>
<th>BIS [μmol]</th>
<th>SPS \textsuperscript{b} [μmol]</th>
<th>Grafting efficiency\textsuperscript{c} [%]</th>
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<td>12.5</td>
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</tr>
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<td>12.5</td>
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<tr>
<td>6</td>
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<td>1.25</td>
<td>12.5</td>
<td>18.6</td>
<td>12.5</td>
<td>105</td>
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\textsuperscript{a} In respect to NiPAAm. \textsuperscript{b} Decomposition of the initiator was induced by TMEDA (6.63 μmol). \textsuperscript{c} The grafting efficiency was determined as described in chapter 4.2, the analytic data is located in the appendix.
5.3 Choice of Macromonomer

As described in chapter 3.5, three different PAAₙ-styrene macromonomers were synthesized varying in the molecular weight (Mₙ = 2600, 4200, and 5700 g/mol). The aim was to identify the ideal molecular weight of the macromonomer for multi-responsive grafted hydrogels. Because temperature response and temperature transition are just slightly affected when the macromonomer content is below 1 mol-% (see chapter 4.3), the influence of the molecular weight on the pH response was exclusively investigated. Figure 5.2 shows the equilibrium swelling degree Qₘ,pH(r.t.) at r.t. for net-PNiPAAm-g-PAAₙ-styrene samples with PAAₙ-styrene graft chains of different molecular weight plotted as a function of pH value. Note that all grafted net-PNiPAAm-g-PAAₙ-styrene hydrogels had a grafting density of 1 mol-%. As expected, the swelling degree of pure net-PNiPAAm prepared in

![Diagram showing equilibrium swelling degree](image)

**Figure 5.2:** (left) Equilibrium swelling degree Qₘ,pH(r.t.) at r.t. for net-PNiPAAm-g-PAAₙ-styrene samples with PAAₙ-styrene graft chains of different molecular weight plotted as a function of pH value. The grafting density of all samples was 1 mol-%. (right) Determined pH response ratio Qₘ,pH(r.t.) for net-PNiPAAm-g-PAAₙ-styrene samples with PAAₙ-styrene graft chains of different molecular weight (color code in the right part of the figure corresponds to the composition of the curves in the left part).
water remains constant with $Q_{m,\text{pH}(r.t.)} \sim 8$ over the investigated pH range (ratio $Q_{m,\text{pH}(r.t.)} \sim 1.1$). The calculated swelling degree is considerably lower compared to $Q_{m,\text{pH}(r.t.)} \sim 40$ of net-PNiPAAm prepared in organic solvent indicating higher mechanical stability. For net-PNiPAAm-$g$-PAA$_n$-styrene, the swelling curves exhibit a transition between pH 4 and pH 8. The swelling degree increases with pH value due to the increased osmotic driving force by the ionized PAA$_n$-styrene graft chains. In comparison to net-PNiPAAm-$g$-PVBA$_{26}$-AAm, the transition range is broadened, which may be attributable to the salt resistivity of PVBA$_{26}$-AAm. Naturally, the osmotic driving force is more pronounced for hydrogels with graft chains of higher molecular weight. This is shown by the ratio $Q_{m,\text{pH}(r.t.)}$ values of 4.6 (net-PNiPAAm-$g$-PAA$_{29}$-styrene), 17.4 (net-PNiPAAm-$g$-PAA$_{53}$-styrene), and 38.1 (net-PNiPAAm-$g$-PAA$_{75}$-styrene). Based on the determined $Q_{m,\text{pH}(r.t.)}$ values, net-PNiPAAm-$g$-PAA$_{53}$-styrene was selected for all following studies due to a good balance between pH response and potentially resulting mechanical stability.

5.4 Response Behavior

pH Response

After the net-PNiPAAm-$g$-PAA$_{53}$-styrene was chosen as the macromonomer, the influence of the grafting density on the pH response was investigated in the next step. Figure 5.3 shows the equilibrium swelling degree $Q_{m,\text{pH}}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities at r.t. (bottom) and 50 °C (top) plotted as a function of pH value. As expected, net-PNiPAAm shows a constant swelling degree and is unaffected by the pH value ($Q_{m,\text{pH}(r.t.)} \sim 8$, $Q_{m,\text{pH}(50 \degree C)} \sim 5$), while the swelling degrees increase as a function of PAA$_{53}$-styrene ionization for net-PNiPAAm-$g$-PAA$_{53}$-styrene. Naturally, the swelling degree of all samples is higher at r.t. compared to 50 °C due to the collapsed PNiPAAm backbone above the VPTT. Employing pH 3 and pH 9 as swollen and collapsed state, the determined ratio $Q_{m,\text{pH}}$ values display an increasing pH response with PAA$_{53}$-styrene content. Whereas net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 1 mol-% PAA$_{53}$-styrene exhibits the highest pH response with 17.4 at r.t. and 18.6 at 50 °C, net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene has the lowest recorded values with 2.1 at r.t. and 1.3 at 50 °C. The good agreement of the pH response between r.t. and 50 °C (e.g. for net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 1 mol-% PAA$_{53}$-styrene: ratio $Q_{m,\text{pH}(r.t.)} = 17.4$ versus ratio $Q_{m,\text{pH}(50 \degree C)} = 18.6$) indicates that the PNiPAAm backbone just slightly affects the pH response of net-PNiPAAm-$g$-PAA$_{53}$-styrene. Consequently, in order to provide sufficient pH response for an adequate volume change at r.t. and at
50 °C (ratio $Q_{m,pH} > 3$), net-PNiPAAm-g-PAA$_{53}$-styrene needs a grafting density above 0.25 mol-%.

Figure 5.3: (left) Equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-g-PAA$_{53}$-styrene samples with different grafting densities at r.t. (bottom) and 50 °C (top) plotted as a function of pH value. (right) Determined pH response ratio $Q_{m,pH}$ for net-PNiPAAm-g-PAA$_{53}$-styrene samples with different grafting densities. In order to achieve adequate pH response, the grafting density must be higher than 0.25 mol-% (color code in the right part of the figure corresponds to the composition of the curves in the left part).[43]

**Temperature Response**

Figure 5.4 presents the equilibrium swelling degree $Q_{m,T}$ for net-PNiPAAm-g-PAA$_{53}$-styrene samples with different grafting densities in pH 3 (top) and pH 9 buffer solution (bottom) plotted as a function of temperature as well as the resulting ratio $Q_{m,T}$. As displayed, the swelling degree of all samples decreases with increasing temperature due to a coil-to-globule transition of the PNiPAAm backbone. In pH 3 buffer solution, when the PAA$_{53}$-styrene graft chains are protonated and hydrophobic, lower swelling degrees are observed with an increasing graft density. For example, pure net-PNiPAAm shows
a drop of the swelling degree from 9 to 3, while the swelling degree of net-PNiPAAm-g-PAA\textsubscript{53}-styrene containing 1 mol-% PAA\textsubscript{53}-styrene decreases from 1.6 to 0.4. Although all samples reveal theoretically suitable temperature response with ratio $Q_{m,T(pH \, 3)} > 3$, it is important to note that net-PNiPAAm-g-PAA\textsubscript{53}-styrene with a graft density > 0.25 mol-% can be used just for some microfluidic applications because of the low swelling degree (i.e. low volume). In pH 9 buffer solution, when the PAA\textsubscript{53}-styrene graft chains are ionized and hydrophilic, the swelling degree increases with PAA\textsubscript{53}-styrene content. However, the determined ratio $Q_{m,T(pH \, 9)}$ shows that the value drops below the prerequisite of 3 at a grafting density of 1 mol-% PAA\textsubscript{53}-styrene. Thus, in order to retain adequate temperature response and to introduce pH response, the grafting density needs to be smaller than 1 mol-%.

![Graph](image-url)

**Figure 5.4:** (left) Equilibrium swelling degree $Q_{m,T}$ for net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities in pH 3 (top) and pH 9 buffer solution (bottom) plotted as a function of temperature. (right) Determined temperature response ratio $Q_{m,T}$ for net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities. In order to retain suitable temperature response, the grafting density must be smaller than 1 mol-% (color code in the right part of the figure corresponds to the composition of the curves in the left part).[^43]
In order to evaluate the temperature response of \textit{net-PNiPAAm-g-PAA}_53-styrene hydrogels in terms of VPTT and transition range, DSC and UV/Vis analysis were carried out. Note that all measurements were conducted with samples equilibrated in distilled water for 24 h. Figure 5.5 displays the determination of the VPTT using the cloud point method.\cite{156} Pure \textit{net-PNiPAAm} prepared in water shows a sharp volume phase transition with a determined VPTT of 34.9 ± 0.1 °C. This value is in comparison to \textit{net-PNiPAAm} synthesized in an organic solvent notably higher (VPTT = 32.4 °C), which may be attributable to the different preparation conditions (organic solvent versus water at pH 10). The DSC thermograph of \textit{net-PNiPAAm} confirms the higher VPTT determined to be 36.4 ± 0.0 °C (Figure 5.5). Unfortunately, UV/Vis and DSC analyses of grafted \textit{net-PNiPAAm-g-PAA}_53-styrene displayed a broader transition with VPTTs shifted to higher values. This result may be caused by the hydrophilic nature of ionized PAA\textsubscript{53}-styrene and increases with the amount of PAA\textsubscript{53}-styrene. Thus, \textit{net-PNiPAAm} exhibits the sharpest transition range from 33 to 36 °C, while \textit{net-PNiPAAm-g-PAA\textsubscript{53}}-styrene containing 1 mol-% PAA\textsubscript{53}-styrene has the broadest transition between 32 and 50 °C. Concerning an application as a chemo-mechanical valve, low grafting density is highly demanded to retain a sharp and discontinuous transition accompanied by a low VPTT.

![DSC and UV/Vis graphs](image)

\textbf{Figure 5.5:} Determined VPTT for \textit{net-PNiPAAm-g-PAA\textsubscript{53}}-styrene samples with different grafting densities in distilled water by (left) DSC and (right) UV/Vis light transmittance using the cloud point method. No onset point was detected in the DSC plot of \textit{net-PNiPAAm-g-PAA}_53-styrene containing 1 mol-% PAA\textsubscript{53}-styrene.\cite{43}
Swelling and Shrinking

As shown in chapter 4.3, net-PNiPAAm-g-PVBA\textsubscript{26}-AAm exhibits a poor swelling-deswelling behavior in pH 3 buffer solution upon temperature changes due to possible π-interactions and H-bonded complexes. Consequently, the swelling-deswelling behavior of net-PNiPAAm-g-PAA\textsubscript{53}-styrene was tested by multiple swelling and shrinking cycles using temperature and pH stimulus.

![Swelling and Shrinking Graph](image)

**Figure 5.6:** (left) Equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities at r.t. (bottom) and 50 °C (top) when the hydrogel was transferred from pH 9 to pH 3 buffer solution, and vice versa. (right) Equilibrium swelling degree $Q_{m,T}$ for net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities in pH 9 (bottom) and pH 3 buffer solution (top) when the temperature of the swelling agent was alternated between r.t. and 50 °C.
Figure 5.6 displays the equilibrium swelling degree $Q_{m,pH}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities at r.t. (bottom) and 50 °C (top) when the swelling agent was alternated between pH 3 and pH 9 buffer solution. At r.t., when the PNiPAAm backbone is swollen, no alternating swelling degree is observed for pure net-PNiPAAm, while net-PNiPAAm-$g$-PAA$_{53}$-styrene shows highly reproducible swelling and shrinking over four consecutive cycles. This emphasizes the high potential of the grafted hydrogels in microfluidic applications. Surprisingly, at 50 °C, when the PNiPAAm backbone is collapsed, pure net-PNiPAAm reveals slightly pronounced swelling and shrinking, which may be caused by the salts of the different buffer solutions.$^{[167–169]}$ For net-PNiPAAm-$g$-PAA$_{53}$-styrene, the swelling degree switches nicely between swollen and collapsed state with more or less constant swelling degrees over the investigated cycles when the pH value was alternated between pH 3 and pH 9.

The swelling and shrinking of net-PNiPAAm-$g$-PAA$_{53}$-styrene and net-PNiPAAm was determined in pH 3 (top) and pH 9 buffer solution (bottom) when the temperature of the swelling agent was changed from r.t. to 50 °C, and vice versa (Figure 5.6 right). In pH 9 buffer solution, thus ionized PAA$_{53}$-styrene graft chains, all samples reveal reproducible swelling and deswelling between r.t. and 50 °C. Changes of the swelling degree were not detected highlighting usability as stimulus. When the PAA$_{53}$-styrene graft chains are protonated in pH 3 buffer solution, shrinking and swelling is repeatable with slight differences between second and third cycle for pure net-PNiPAAm and net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene. This result may be contributed to a skin effect known for net-PNiPAAm hydrogels.$^{[95,170]}$ In summary, temperature and pH response show great potential as stimuli in microfluidics due to the highly reproducible swelling and shrinking properties of the net-PNiPAAm-$g$-PAA$_{53}$-styrene hydrogels.

**Salt Response**

High salt concentration leads to partial water release of net-PNiPAAm-$g$-PVBA$_{26}$-AAm hydrogels as described in chapter 4.3. Consequently, salt equilibrium swelling studies were performed with net-PNiPAAm-$g$-PAA$_{53}$-styrene hydrogels as well. NaCl was selected as the model salt because it is readily available and inexpensive. Note that additional shrinking is provoked by the compensation of the charged PAA side groups and reduction of the resulting osmotic pressure. Figure 5.7 displays the equilibrium swelling degree $Q_{m,salt}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities plotted as a function of NaCl concentration.

For pure net-PNiPAAm, the swelling degree is constant between 0 and 0.1 mol/L with $Q_{m,NaCl} \approx 10$. At higher NaCl concentration ($c > 0.1$ mol/L), the swelling degree drops
Figure 5.7: (left) Equilibrium swelling degree $Q_{m,NaCl}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities plotted as a function of NaCl concentration. (right) Determined NaCl response ratio $Q_{m,NaCl}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities. The NaCl response of all samples is suitable for an application as a chemo-mechanical valve (color code in the right part of the figure corresponds to the composition of the curves in the left part).

to ~ 3. In contrast, the swelling degree of net-PNiPAAm-$g$-PAA$_{53}$-styrene is constant until 0.001 mol/L NaCl and decreases then linearly with NaCl concentration independent of the grafting density. The determined NaCl response reveals a non-linear relationship between grafting density and response. Grafted net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.5 mol-% PAA$_{53}$-styrene shows the highest salt response with ratio $Q_{m,NaCl} = 4.9$, whereas pure net-PNiPAAm exhibits the lowest salt response calculated to be 3.1. But even so, all samples fulfill the prerequisite of a ratio $Q_{m,NaCl} > 3$ indicating suitable NaCl response for an application as a chemo-mechanical valve. It is worth noting that repeated cycling between swollen and collapsed state was evaluated in terms of usability as a stimulus. As shown in Figure 5.9, the hydrogel samples were transferred from distilled water to 1 M NaCl solution, and vice versa over four consecutive cycles. All samples showed more or less constant swelling degrees between swollen and collapsed state.

Solvent Response

Like for net-PNiPAAm-$g$-PVBA$_{26}$-AAm, the solvent response was investigated for net-PNiPAAm-$g$-PAA$_{53}$-styrene as well. It should be mentioned that the model system ethanol/water was used because several studies have employed ethanol/water mixtures to expand and contract hydrogels in microfluidic devices.$^{[44,171]}$
Figure 5.8: (left) Equilibrium swelling degree $Q_{m,\text{EtOH}}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities plotted as a function of volume fraction of ethanol in water. (right) Determined solvent response ratio $Q_{m,\text{EtOH}}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities. The ethanol response for all samples is suitable for an application as a chemo-mechanical valve (color code in the right part of the figure corresponds to the composition of the curves in the left part).

Figure 5.8 shows the equilibrium swelling degree $Q_{m,\text{EtOH}}$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples with different grafting densities plotted as a function of volume fraction of ethanol in water. In pure water, all samples are swollen with increasing swelling degree with PAA$_{53}$-styrene content. net-PNiPAAm and net-PNiPAAm-$g$-PAA$_{53}$-styrene collapse at 20 vol-% ethanol and have the lowest swelling degrees around 40 vol-% ethanol. At about 50 vol-% ethanol, net-PNiPAAm and net-PNiPAAm-$g$-PAA$_{53}$-styrene swell again with a maximum swelling degree around 70 vol-% followed by a decreasing swelling degree. The determined ethanol response reveals a non-linear relationship between grafting density and ratio $Q_{m,\text{EtOH}}$ values. While net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene shows the highest determined value of 17.4, net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 1 mol-% PAA$_{53}$-styrene reveals the lowest ratio $Q_{m,\text{EtOH}}$ value with 5.6. This result is contributed to the high deswelling of net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene at the collapsed state. Independently, net-PNiPAAm and net-PNiPAAm-$g$-PAA$_{53}$-styrene have an adequate ethanol response for microfluidics with ratio $Q_{m,\text{NaCl}}$ values $>3$. It should be mentioned that the reproducibility of the swelling and shrinking was tested by alternating the swelling agent between distilled water and 40 vol-% EtOH solution over repeated cycles. As shown in Figure 5.9, the high reproducibility of the swelling degrees indicates that the solvent stimulus is suitable for an application in microfluidics.
5.5 Cooperative Diffusion Coefficient

An important key characteristic of a hydrogel is the rate of the volume phase transition, which is depicted by the response time of a hydrogel. Naturally, a short response time of the active material is highly demanded to provide accelerated opening and closing function as a chemo-mechanical valve. It is worth noting that spherical particles provide uniform expansion and contraction, whereas other geometries such as disk-, worm-, or square-shaped hydrogel particles exhibit prevalently non-uniform swelling and shrinking properties particularly for large particles. For this reason, many applications as a chemo-mechanical valve are based on spherical hydrogel particles. As can be seen from equation 1.3, two parameters enhance the volume phase transition: (i) downscaling due to smaller hydrogel particles and (ii) improved cooperative diffusion coefficients. Because downscaling is exclusively a technological issue, the purpose of this study was to evaluate the $D_{coop}$ values of the prepared net-PNiPAAm-g-PAA₅₃-styrene hydrogels and to compare them with the $D_{coop}$ value of pure net-PNiPAAm. Two methods are known to determine the cooperative diffusion coefficient, either by scattering experiments, e.g. dynamic light scattering.[172–174]
or by the collective diffusion model developed by Tanaka and Fillmore.\cite{124,175} However, the model of Tanaka and Fillmore is exclusively applicable to spherical hydrogel particles. Fortunately, Krause et al. recently reported that the collective diffusion model extended by a volume specific surface can be used to determine the cooperative diffusion coefficient of other hydrogel geometries such as worm- and disk-shaped particles at any aspect ratio.\cite{125} Figure 5.10 shows the corrected $D_{\text{coop}}$ values calculated with the extended model. Furthermore, based on the corrected $D_{\text{coop}}$ values and equation 1.3, the response time $\tau$ of a spherical hydrogel particle with the characteristic dimension of $l = 100 \ \mu m$ was prognosticated. The aim of this prediction is to visualize the effect of the PAA$_{53}$-styrene graft chains on the swelling rate of the hydrogel. Note that all swelling experiments were conducted three times with cylinder-shaped hydrogel samples by swelling from 50 °C to room temperature in pH 9 buffer solution.

![Graph](image)

**Figure 5.10:** Corrected cooperative diffusion coefficient $D_{\text{coop}}$ and response time $\tau$ for net-PNiPAAm-$g$-PAA$_{53}$-styrene samples plotted as a function of the grafting density of PAA$_{53}$-styrene. The response time was calculated with equation $\tau = (l^2)/(\pi^2 \cdot D_{\text{coop}})$ and a characteristic dimension $l = 100 \ \mu m$. Error bars represent the standard deviation ($n = 3$).\cite{43,125}

In the case of pure net-PNiPAAm, a corrected $D_{\text{coop}}$ value of $4.35 \pm 0.69 \cdot 10^{-7}$ cm$^2$/s was estimated, which shows a good accordance with the literature value of $3.2 \cdot 10^{-7}$ cm$^2$/s reported by Tanaka and Fillmore.\cite{124} The slight difference between both values may be attributable to the different net-PNiPAAm compositions and solutions used in the respective experiments. Furthermore, the prognosticated response time of a spherical net-PNiPAAm particle with the characteristic dimension $l = 100 \ \mu m$ was calculated to be $23.8 \pm 2.7 \ s$ ($\tau = 0.01 \ cm^2/(\pi^2 \cdot 4.35 \pm 0.48 \cdot 10^{-7} \ cm^2/s)$). Remarkably, grafted net-PNiPAAm-$g$-PAA$_{53}$-styrene hydrogels reveal higher corrected $D_{\text{coop}}$ values compared to pure net-PNiPAAm. These corrected $D_{\text{coop}}$ values increases from $1.55 \pm 0.30 \cdot 10^{-6}$ (0.25 mol-% PAA$_{53}$-styrene)
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to 2.34 $\pm$ 0.40 $\times$ 10$^{-6}$ (0.5 mol-% PAA$_{53}$-styrene) to 3.95 $\pm$ 0.15 $\times$ 10$^{-6}$ cm$^2$/s (1 mol-% PAA$_{53}$-styrene) with PAA$_{53}$-styrene content. In accordance with literature reports, this tendency is caused by the increasing osmotic pressure of the deprotonated acid groups within the graft chains.$^{[125,176]}$ Naturally, the prognosticated response time of spherical hydrogel particles decreases from 6.5 $\pm$ 1.3 (0.25 mol-% PAA$_{53}$-styrene) to 4.4 $\pm$ 0.9 (0.5 mol-% PAA$_{53}$-styrene) to 2.6 $\pm$ 0.1 s (1 mol-% PAA$_{53}$-styrene) accelerating the closing time of the valve by a factor of 3.7 (0.25 mol-% PAA$_{53}$-styrene), 5.4 (0.5 mol-% PAA$_{53}$-styrene) and 9.2 (1 mol-% PAA$_{53}$-styrene) compared to pure net-PNiPAM. Note that the prognosticated response time of the hydrogels is in the same order of magnitude as the closing time of a net-poly(sodium acrylate) valve employed in a fluidic microchemo-mechanical integrated circuit (size: 500 $\times$ 500 $\times$ 100 $\mu$m, closing time: 45 s).$^{[177]}$ This additionally highlights the good agreement between prediction by the extended collective diffusion model and application in a microfluidic device.

5.6 Mechanical Stability

Like for net-PNiPAM-$g$-PVBA$_{26}$-AAm hydrogels, the mechanical properties of grafted net-PNiPAM-$g$-PAA$_{53}$-styrene hydrogels were evaluated employing rheological analysis. As done before, all samples were equilibrated in pH 9 buffer solution for 24 h to ensure that the hydrogels are totally swollen.

Figure 5.11 shows the storage modulus at an angular velocity of $\omega = 1$ rad/s and the swelling degree plotted as a function of the PAA$_{53}$-styrene content. For pure net-PNiPAM hydrogel, the highest storage modulus, precisely 37.2 $\pm$ 2.9 kPa, was measured. The storage modulus of grafted net-PNiPAM-$g$-PAA$_{53}$-styrene hydrogels, hence their mechanical stability, decreases with increasing grafting density of PAA$_{53}$-styrene. Thus, net-PNiPAM without PAA$_{53}$-styrene graft chains exhibits the best mechanical properties. It is worth noting that net-PNiPAM-$g$-PAA$_{53}$-styrene hydrogels containing 0.5 mol-% and 1 mol-% PAA$_{53}$-styrene reveal storage moduli in the same order of magnitude (10.1 $\pm$ 0.2 and 9.5 $\pm$ 0.3 kPa), indicating that the storage modulus is inversely proportional to the PAA$_{53}$-styrene content and swelling degree, respectively. Other literature reports validate this result.$^{[166,178]}$ Importantly, all samples maintained their disk-shaped geometry without damage by the compression and shear forces during the rheological analysis. This result highlights superior mechanical properties of net-PNiPAM-$g$-PAA$_{53}$-styrene compared to net-PNiPAM-$g$-PVBA$_{26}$-AAm. In summary, net-PNiPAM-$g$-PAA$_{53}$-styrene hydrogels exhibit considerably better mechanical properties compared to net-PNiPAM-$g$-PVBA$_{26}$-AAm hydrogels. However, the amount of PAA$_{53}$-styrene should be highly oriented
towards the mechanical stress in the microfluidic application and low grafting density is recommended to provide best possible mechanical properties and to ensure long-term stability.

Figure 5.11: The storage modulus and swelling degree $Q_{m,pH\,9}$ at r.t. for net-PNiPAAm-g-PAA$_{53}$-styrene samples plotted as a function of the grafting density of PAA$_{53}$-styrene. Error bars represent the standard deviation ($n = 3$).

5.7 Photopolymerization

The main technique to fabricate microfluidic systems with complex micropatterns is soft lithography. Soft lithography offers the advantages of a rapid, simple, and low-cost fabrication method. Accordingly, photopolymerization and photopatterning of hydrogels are highly desired to polymerize hydrogels directly inside microfluidic channels and to use exclusively one method to fabricate microfluidic devices with incorporated hydrogels. Note that ex situ fabrication such as radical polymerization requires manual incorporation of the hydrogel in the microfluidic devices, which is particularly difficult for smaller dimensions. For this reason, the polymerization of grafted net-PNiPAAm-g-PAA$_{53}$-styrene was investigated using photoinitiation. Importantly, all polymerizations were conducted using the same feed ratio of NiPAAm, PAA$_{53}$-styrene, BIS, and initiator as described in chapter 5.2. As a useful photoinitiator for this experiment, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was prepared according to literature (Figure 5.12). LAP provides high polymerization rate as well as good water solubility. It is worth noting that the gelation time in this experiment is defined as the required time to turn a 0.1 ml feed solution to a solid gel with 320 - 500 nm wavelength light (light intensity: 0.3 W/cm$^2$, 1%).
Figure 5.12 shows the gelation time plotted as a function of the molar amount of PAA$_{53}$-styrene. Interestingly, the gelation time increases from 20 to 140 s with PAA$_{53}$-styrene content indicating a linear relationship between both parameters. This result is in good agreement with literature reports showing that polymerization rate $R_p$ is proportional to double bond [DB] as well as initiator concentration [I] ($R_p \sim [DB][I]^{1/2}$). Accordingly, a decrease in the initiator and double bond concentration, which occurs with increasing PAA$_{53}$-styrene content, leads to lower rates of polymerization and consequently, longer gelation time. Remarkably, all experiments were conducted at 10 °C under ambient conditions without protective gas atmosphere highlighting the simplicity of preparing grafted net-PNiPAAm-g-PAA$_{53}$-styrene via photopolymerization.

**Figure 5.12:** (left) Gelation time for net-PNiPAAm-g-PAA$_{53}$-styrene samples plotted as a function of the grafting density of PAA$_{53}$-styrene. (right) Feed solution before and after a photopolymerization.

### 5.8 Chapter Summary

Within this chapter, grafted net-PNiPAAm-g-PAA$_n$-styrene hydrogels with a PNiPAAm backbone and PAA$_n$-styrene graft chains were prepared and characterized. Because net-PNiPAAm-g-PAA$_{53}$-styrene revealed adequate pH response, all following studies were conducted with this macromonomer. ATR-IR studies using the baseline method indicated high grafting density of all net-PNiPAAm-g-PAA$_n$-styrene hydrogels with values > 90 %. This implied a good accessible styrene end-group and quantitative incorporation of PAA$_n$-styrene into the PNiPAAm backbone.

The main goal was to identify suitable stimuli for an application as a chemo-mechanical valve and to show reversibility of the swelling and shrinking process. Importantly, the
temperature sensitivity had to be retained, while a pH response needed to be introduced. Equilibrium swelling studies quantified with the response ratio $Q_{m,i}$ revealed that a grafting density of PAA$_{53}$-styrene between 0.25 and 1 mol-% provides a suitable response towards temperature, pH, salt, and solvent. Furthermore, the swelling and shrinking process for all four stimuli was highly reproducible for four consecutive cycles (Figure 5.13).

In order to evaluate the swelling kinetics of grafted net-PNiPAm-g-PAA$_{53}$-styrene hydrogels, the collective diffusion model extended by a volume specific surface was applied.\cite{125}

### Table 5.2: Grafting efficiency, VPTT, corr. $D_{coop}$, response time, storage modulus, and gelation time for net-PNiPAm-g-PAA$_{53}$-styrene hydrogels with different grafting densities.

<table>
<thead>
<tr>
<th>PAA$_{53}$-styrene</th>
<th>Grafting efficiency$^a$</th>
<th>VPTT$^{a,b}$</th>
<th>corr. $D_{coop}^a$</th>
<th>Response time$^a$</th>
<th>Storage modulus$^a$</th>
<th>Gelation time$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[mol-%]</td>
<td>[%]</td>
<td>[°C]</td>
<td>[cm$^2$/s]</td>
<td>[s]</td>
<td>[kPa]</td>
<td>[s]</td>
</tr>
<tr>
<td>0</td>
<td>-</td>
<td>34.9 ± 0.1</td>
<td>4.4 ± 0.7·10$^{-7}$</td>
<td>23.8 ± 2.7</td>
<td>37.2 ± 1.3</td>
<td>20</td>
</tr>
<tr>
<td>0.25</td>
<td>91</td>
<td>36.4 ± 0.1</td>
<td>1.6 ± 0.3·10$^{-6}$</td>
<td>6.5 ± 1.3</td>
<td>19.7 ± 0.9</td>
<td>35</td>
</tr>
<tr>
<td>0.5</td>
<td>98</td>
<td>41.3 ± 0.2</td>
<td>2.3 ± 0.4·10$^{-6}$</td>
<td>4.4 ± 0.9</td>
<td>10.1 ± 0.2</td>
<td>70</td>
</tr>
<tr>
<td>1</td>
<td>94</td>
<td>43.0 ± 2.2</td>
<td>4.0 ± 0.2·10$^{-6}$</td>
<td>2.6 ± 0.1</td>
<td>9.5 ± 0.3</td>
<td>140</td>
</tr>
</tbody>
</table>

$^a$ Properties of net-PNiPAm-g-PAA$_{53}$-styrene prepared via FRP as described in chapter 5.2. $^b$ Determined in water by UV/Vis using the cloud point method.\cite{156} $^c$ Gelation time in a photopolymerization as described in chapter 5.7.
The determined cooperative diffusion coefficients of \textit{net-PNiPAAm-g-PAA\textsubscript{53}-styrene} and \textit{net-PNiPAAm} indicated faster response time with increasing PAA\textsubscript{53}-styrene content. Remarkably, \textit{net-PNiPAAm-g-PAA\textsubscript{53}-styrene} exhibited an accelerated swelling rate by a factor of 9 compared to pure \textit{net-PNiPAAm}. As it could be expected, the rheological analysis showed that an increasing graft density leads to decreasing mechanical stability. However, grafted \textit{net-PNiPAAm-g-PAA\textsubscript{53}-styrene} revealed higher mechanical strength compared to \textit{net-PNiPAAm-g-PVBA\textsubscript{26}-AAm} suitable for an application in microfluidics. Another important aspect was to evaluate the photopolymerization of \textit{net-PNiPAAm-g-PAA\textsubscript{53}-styrene}. It was found that the gelation time linearly increases with PAA\textsubscript{53}-styrene grafting density. Table 5.2 summarizes the results of this chapter.
6 Grafted Hydrogels for Flow Control

6.1 Introduction

Valves are indispensable in many microfluidic applications to regulate the fluid flow. Employing hydrogels as a chemo-mechanical valve is particularly attractive for autonomous flow control because they work self-sufficiently and are independent of external controls. The gel autonomously detects an environmental condition and changes its state accordingly. Therefore, it works as a sensor and an actuator simultaneously. As shown in the last chapters, grafted net-PNiPAAm-g-PAA\textsubscript{53}-styrene hydrogels exhibit the desired properties for an application as a chemo-mechanical valve in microfluidics (chapter 5), whereas net-PNiPAAm-g-PVBA\textsubscript{26}-AAm reveals extremely poor mechanical properties unsuitable for microfluidic systems with any mechanical stress (chapter 4). Consequently, net-PNiPAAm-g-PAA\textsubscript{53}-styrene was exclusively used to prove the concept of grafted hydrogels as a chemo-mechanical valve. Two applications of grafted hydrogels as a chemo-mechanical valve will be discussed. The first one is based on a straightforward fluidic set-up in which an actuator chamber encloses the hydrogel. The other valve is characterized by an individual channel for the hydrogel. In both set-ups, the hydrogel volume (i.e. swelling degree) directs the flow rate. Because four stimuli were identified to be suitable for a usage, all four stimuli were tested in terms of feasibility in the respective valve. On top of that, consecutively employing of different stimuli in one experiment was aimed. Because many microfluidic applications demand an equal response to different stimuli, the grafting density for an equal temperature and pH response was calculated as well as tested in a valve. Most results of chapter 6 are submitted to *ACS Applied Materials & Interfaces*, 29-Mar-2016 and close to submission for *Nature Communication*.[43,45]

6.2 Tetra-Responsive Chemo-Mechanical Valve

A fluidic test station was constructed to prove the concept of grafted hydrogels as a chemo-mechanical valve. This test station is particularly interesting for microfluidics due to the simplicity of the set-up. Other important advantages are that the mechanical stability
of the hydrogel is indifferent and crushed hydrogel particles can be used. Moreover, the set-up benefits from the small size of the crushed particles, which additionally accelerates the rate of the volume phase transition accompanied by a fast opening and closing of the valve.

![Diagram of fluidic test station](image)

**Figure 6.1:** (left) Schematic design of the fluidic test station: a fluid reservoir, an inlet channel, an actuator chamber filled with crushed hydrogel particles, and an outlet channel. (right) Operating principle of the actuator chamber filled with hydrogel particles: the swelling degree of the enclosed hydrogel directs whether the valve is opened or closed (chamber size 10 × 1 or 10 × 4 mm). The arrows denote the direction of the fluid flow.\[^{[43]}\]

Figure 6.1 presents the schematic design of the fluidic test station. The set-up comprises (i) a fluid reservoir, (ii) an inlet channel, (ii) a cylinder-shaped actuator chamber filled with crushed hydrogel particles, and (iv) an outlet channel. The cylinder-shaped actuator chamber filled with crushed hydrogel particles was designed in reference to literature reports.\[^{[17]}\] Crushed hydrogel particles are enclosed between two filter plates. The filter plates (pore size 160 - 250 $\mu$m) permanently enclose the hydrogel particles within the actuator chamber, whereas fluid can flow through the porous plates of the valve. Therefore, the active resistance of the hydrogel particles in the actuator chamber directs the flow rate. This allows flow control by the local environmental conditions. When the hydrogel is swollen (i.e. in pH 9 buffer solution at r.t.), the hydrogel completely fills the actuator chamber and throttles or shut offs the fluid flow in the flow channel. On the contrary, when the hydrogel is collapsed (e.g. in pH 3 buffer solution at r.t.), the hydrogel particles just partially fill the actuator chamber and the fluid can easier flow through the chamber resulting in a decreasing fluidic resistance accompanied by a higher flow rate. Note that
the same solvents were taken as in the response studies to expand or to contract the hydrogel particles.

It should be mentioned that the employed filter plates are mainly composed of silicon dioxide (~ 80 %), which is known to interact with polar as well as non-polar solvents.[182,183] In order to analyze the interactions between the filter plate and fluid, a control experiment with the fluidic test station and an empty actuator chamber was conducted. Figure 6.2 shows the uncorrected flow rate of each fluid (e.g. pH 9 = pH 9 buffer solution, 50 °C = pH 9 buffer solution at 50 °C, pH 3 = pH 3 buffer solution, EtOH = 40 vol-% EtOH solution, NaCl = 1 mol/L NaCl solution). The uncorrected flow rate was quantified by determining the time for 10 ml outflow (error bars represent the standard deviation, n = 5). Note that the fluid reservoir is refilled for each trial to provide equal pressure from the inlet channel.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Uncorr. Flow Rate [ml/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 9</td>
<td>0.4</td>
</tr>
<tr>
<td>pH 3</td>
<td>0.86</td>
</tr>
<tr>
<td>EtOH</td>
<td>2.3</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.89</td>
</tr>
<tr>
<td>50 °C</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Figure 6.2: Uncorrected flow rate of different fluids using the fluidic test station with an empty actuator chamber. The determined correction factor $c_i$ of each fluid is shown above the bar (pH 9 = pH 9 buffer solution at r.t., 50 °C = pH 9 buffer solution at 50 °C, pH 3 = pH 3 buffer solution at r.t., NaCl = 1 mol/L NaCl solution, EtOH = 40 vol-% ethanol solution). Error bars represent the standard deviation (n = 5).[43]

As it could be expected, the evaluated fluids reveal different flow rates. For example, a 40 vol-% ethanol solution shows the lowest recorded flow rate with $\dot{V}_{\text{EtOH}} = 0.18$ ml/s, while pH 9 buffer solution at 50 °C exhibits the highest flow rate calculated to be $\dot{V}_{50 \, ^\circ \text{C}} = 0.6$ ml/s. In order to extinguish the effect of the fluid and to equal the flow rate, the correction factor $c_i$ is introduced:

$$c_i = \frac{\dot{V}_{\text{pH \, 9}}}{\dot{V}_i},$$

(6.1)

where $\dot{V}_{\text{pH \, 9}}$ is the flow rate with pH 9 buffer solution (i.e. $\dot{V}_{\text{pH \, 9}} = 0.4$ ml/s) and $\dot{V}_i$ is the uncorrected flow rate of a fluid (e.g. for the pH stimulus a pH 3 buffer solution).
Figure 6.2 displays the determined correction factors $c_i$ for each fluid above the bar (e.g. $c_{pH\,3} = \frac{0.4 \text{ ml/s}}{0.465 \text{ ml/s}} = 0.86$). Consequently, the corrected flow rate $\dot{V}_{c,i}$ is defined as follows:

$$\dot{V}_{c,i} = \dot{V}_i \cdot c_i.$$  \hspace{1cm} (6.2)

Exemplarily, the corrected flow rate of a pH 3 buffer solution is determined by $\dot{V}_{c,pH\,3} = 0.465 \text{ ml/s} \cdot 0.86 = 0.4 \text{ ml/s}$ and is equal to the corrected flow rate of pH 9 buffer solution. It is worth noting that a plot with corrected as well as uncorrected flow rate applying different stimulus is presented in the appendix (Figure 9.9).

![Figure 6.3: Optical microscope images of net-PNiPAAm-g-PAA\textsubscript{53}-styrene containing 1 mol\%-PAA\textsubscript{53}-styrene](image)

As shown in the equilibrium swelling studies of net-PNiPAAm-g-PAA\textsubscript{53}-styrene, a grafting density between 0.25 and 1 mol\%- is essential to achieve a sufficient volume change towards pH, temperature, solvent, and salt stimulus (ratio $Q_{m,i} > 3$). However, it is necessary to provide equal response independent of the applied stimulus in many microfluidic applications. Consequently, the PAA\textsubscript{53}-styrene content for an equal temperature and pH response was calculated in the next step. For this purpose, the macroscopic diameter change of disk-shaped net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities after pH or temperature stimulus was determined using optical microscopic images. Note that all samples were 24 h equilibrated at the condition of interest. Exemplarily, Figure 6.3 presents three images of net-PNiPAAm-g-PAA\textsubscript{53}-styrene containing 1 mol\%- PAA\textsubscript{53}-styrene: one in the initial state when the hydrogel is totally swollen (middle, in pH 9 buffer solution at r.t.), and two when the hydrogel is partially collapsed after pH (right, in pH 3 buffer solution at r.t.) or temperature stimulus (left, in pH 9 buffer solution at 50 °C). The diameter change $\Delta d_i$ was calculated using the following equation:
where \( d_0 \) is the diameter of the disk-shaped sample before the stimulus and \( d_i \) is the diameter of the disk-shaped sample after a stimulus. According to equation 6.3, a diameter change of 54 % by pH stimulus (\( d_0 = 5.85 \text{ mm}, d_{\text{pH}} = 2.69 \text{ mm} \)) and 18.5 % by temperature stimulus (\( d_0 = 5.85 \text{ mm}, d_{\text{T}} = 4.77 \text{ mm} \)) was calculated for \( \text{net-PNiPAAm-}g\text{-PAA}_{53}\text{-styrene} \) containing 1 mol-% PAA\(_{53}\)-styrene.

**Figure 6.4:** Diameter change \( \Delta d \) for \( \text{net-PNiPAAm-}g\text{-PAA}_{53}\text{-styrene} \) samples after applying pH or temperature stimulus plotted as a function of the grafting density.\(^{43}\)

Figure 6.4 shows the diameter change of \( \text{net-PNiPAAm-}g\text{-PAA}_{53}\text{-styrene} \) samples after pH or temperature stimulus plotted as a function of the grafting density. In agreement with the equilibrium swelling studies in chapter 5.4, the temperature stimulus shows a linear decrease in diameter change from 53 to 19 % with increasing grafting density, while the diameter change increases linearly from 2 to 54 % with increasing PAA\(_{53}\)-styrene content by the pH stimulus. The intercrossing point of both slopes directs the PAA\(_{53}\)-styrene content for an equal pH and temperature response, which turned out to be 0.6 mol-% PAA\(_{53}\)-styrene. Importantly, the determined PAA\(_{53}\)-styrene content fulfills the prerequisite of a stimuli response ratio \( Q_{m,i} > 3 \) because it is between 0.25 and 1 mol-% PAA\(_{53}\)-styrene. \( \text{Net-PNiPAAm-}g\text{-PAA}_{53}\text{-styrene} \) containing 0.6 mol-% PAA\(_{53}\)-styrene was prepared as depicted in chapter 5.2 and revealed a grafting efficiency of 96 %.

In the next step, a cylinder-shaped actuator chamber of \( 10 \times 4 \text{ mm} \) (diameter \( \times \) height) was filled with crushed hydrogel particles of \( \text{net-PNiPAAm-}g\text{-PAA}_{53}\text{-styrene} \) containing 0.6 mol-% PAA\(_{53}\)-styrene and the reversibility of the opening and closing function was tested. In a typical procedure, the fluid reservoir was filled with the fluid of interest and...
the flow rate was determined by stopping the time for 10 ml outflow. Thus, all flow rate graphs in this chapter are plotted as a function of outflow and not, as usual, as a function of time. Note that the flow rate was corrected using the determined correction factor $c_i$. Figure 6.5 displays the corrected flow rate plotted as a function of the outflow when the temperature of the pH 9 buffer solution in the fluid reservoir was alternated between r.t. and 50 °C. As shown, swelling and deswelling of the grafted hydrogel can regulate the fluid flow in the flow channel. No fluid flow is detected when the inlet is provided with a pH 9 buffer solution at r.t. because the swollen hydrogel particles shut off the flow channel and prevent a fluid flow. On the contrary, the valve rapidly opens in less than seconds when the temperature of pH 9 buffer solution changes to 50 °C. If needed, even faster opening of the chemo-mechanical valve could be obtained by adjusting the particle size.\textsuperscript{110} Interestingly, the flow rate increases during the first two cycles and is then constant at around 0.27 ml/s over the remaining four cycles. In reference to literature, this may be provoked by a conditioning effect due to the swelling and shrinking process.\textsuperscript{171}

![Graph showing corrected flow rate as a function of outflow with temperature and pH 9 buffer solution conditions.](image)

**Figure 6.5:** Corrected flow rate plotted as a function of the outflow. The temperature of the pH 9 buffer solution was alternated between r.t. and 50 °C. The color background of the diagram represents the provided solution. An actuator chamber with a size of $10 \times 4$ mm filled with crushed particles of net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene was used in this experiment.\textsuperscript{43}

It should be mentioned that the opening of the actuator chamber with a size of $10 \times 4$ mm took place relatively slowly when the pH, salt, or solvent stimulus was used. This is due to the different transfer coefficients, which is two order of magnitudes larger for heat than for mass ($D_H \approx 10^{-3}$ cm$^2$/s $> D_M \approx 10^{-5}$ cm$^2$/s).\textsuperscript{171} In order to accelerate the opening time of the actuator chamber, a second fluidic test station using a smaller cylinder-shaped actuator chamber with a size of $10 \times 1$ mm was designed. Figure 6.6 shows
the corrected flow rate plotted as a function of the outflow when the solution in the fluid reservoir was alternated between pH 9 buffer solution at r.t. and one solution in which the hydrogel collapses (e.g. pH 3 buffer solution, pH 9 buffer solution at 50 °C, 40 vol-% EtOH solution, or 1 mol/L NaCl solution). Remarkably, a 1 mm layer of crushed hydrogel particles can already regulate the fluid flow. Note that each stimulus was investigated in an individual experiment. When the inlet channel is provided with a pH 9 buffer solution at r.t., the flow rate is considerably throttled between 0.5 and 1.5 ml/s. At these conditions, the hydrogel particles are swollen and fill the actuator chamber completely.

Figure 6.6: Corrected flow rate plotted as a function of the outflow. The solution was alternated between pH 9 buffer solution at r.t. and one solution in which the hydrogel collapses (e.g. pH 3 buffer solution, pH 9 buffer solution at 50 °C, 40 vol-% EtOH solution, or 1 mol/L NaCl solution). The color background of the diagram represents the provided solution. An actuator chamber with a size of 10 × 1 mm filled with crushed particles of net-PNiPAAm-g-PAA₅₃-styrene containing 0.6 mol-% PAA₅₃-styrene was used in this experiment.⁴³
However, because the hydrogel layer is thin, the actuator chamber just throttles the fluid flow without completely clogging the flow channel. This has the additional advantage that the actuator chamber is permanently provided with fresh inlet solution without a bypass. After changing the fluid of the inlet to one in which the hydrogel collapses (pH 3 buffer solution, pH 9 buffer solution at 50 °C, 40 vol-% EtOH solution, or 1 mol/L NaCl solution), the flow rate immediately increases about ~ 2 - 2.5 times. It should be noted that different actuator chambers were used for each stimulus leading to slight differences in the corrected flow rates. Importantly, closing and opening of the chemo-mechanical valve is repeatable over 5 consecutive cycles. However, the corrected flow rate partially shifts to higher values. As mentioned before, this is contributed to a conditioning effect. For the ethanol response, the opening and closing function is not reproducible. In this case, small air bubbles were observed in the actuator chamber, which may be caused by the side reaction of ethanol and residual borax of the pH 9 buffer solution ($\text{B}_4\text{O}_7^{2-} + 5\text{H}_2\text{O} + 2\text{H}^+ \rightarrow 4\text{B(OH)}_3$). However, further research is needed for full understanding.

![Graph](image)

**Figure 6.7**: Corrected flow rate plotted as a function of the outflow. The solution was changed between pH 9 buffer solution at r.t. and one solution in which the hydrogel collapses. The solution in which the hydrogel collapses was alternated between pH 9 buffer solution at 50 °C, pH 3 buffer solution, and 1 mol/L NaCl solution. The color background of the diagram represents the provided solution. An actuator chamber with a size of $10 \times 1$ mm filled with crushed particles of *net-PNiPAAm-g-PAA$_{53}$-styrene* containing 0.6 mol-% PAA$_{53}$-styrene was used in this experiment.

In order to outline the advantageous properties of *net-PNiPAAm-g-PAA$_{53}$-styrene* containing 0.6 mol-% PAA$_{53}$-styrene as a chemo-mechanical valve, the reversibility of the opening and closing function was demonstrated by employing three different stimuli in a
consecutive way (i.e. temperature, pH, and salt). The ethanol response was excluded for this experiment because of the side reaction of ethanol and pH 9 buffer solution leading to inconstant flow rates. As shown in Figure 6.7, the fluid flow is throttled between 0.15 and 0.2 ml/s due to the swollen hydrogel particles within the actuator chamber when pH 9 buffer solution at r.t. was provided. On the contrary, when a solution was added in which the hydrogel collapses, the valve immediately opens and the corrected flow rate rises by a factor of two. Importantly, the opening and closing function is reversible over six cycles even when three different stimuli were employed consecutively. It should be outlined that the change in fluid flow is more or less equal for all three stimuli. This remarkably demonstrates high control of the flow rate as well as an equal temperature, pH value, and salt response of net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene.

### 6.3 Chemo-Fluidic Membrane Transistor

As shown in the last chapter, net-PNiPAAm-g-PAA$_{53}$-styrene can be used for flow control. However, the presented fluidic test station with the hydrogel in the flow channel requires that all components, such as an analyte in the fluid, are compatible with the applied stimulus (e.g. pH, temperature, as examples). In order to broaden potential applications in microfluidics, alternative valve designs are highly demanded. Recently, Frank et al. reported of a chemo-fluidic membrane transistor (CFMT) in which hydrogel particle and flow channel are separated by a thin flexible membrane.$^{[44,46]}$ A detailed description of the operating principle can be found in chapter 1.4. The transistor based on a thin PDMS membrane (~ 30 μm) sandwiched between two layers in a crossed-channel architecture.

The upper layer contains the flow channel with a channel barrier, while the bottom layer comprises the control channel with the hydrogel particle. Note that the channel barrier is located exactly over the hydrogel particle, which is held in place by a cage of posts. Like in the fluidic test station discussed in chapter 6.2, the swelling degree of the hydrogel directs the flow rate in the flow channel. When the hydrogel is collapsed and pressure is applied in the flow channel, the valve opens due to deflection of the membrane downward resulting in a flow in the upper channel. In contrast, when the hydrogel is swollen, the particle puts pressure on the membrane and keeps it in place even at high pressure in the flow channel. As part of a collaboration project with the chair of polymeric microsystems within the Cluster of Excellence Center for Advancing Electronics Dresden (A. Richter and P. Frank), membrane assures hydraulic coupling in CFMT technology and grafted net-PNiPAAm-g-PAA$_{53}$-styrene hydrogels were combined to emphasize the potential of both systems.
The CFMT was fabricated with P. Frank according to literature. As shown in Figure 6.8, two master molds were prepared for making the flow and control layer. In a typical procedure, (i) a clean glass substrate was laminated with a photo resist and (ii) UV radiated through a photo mask. (iii) After baking both master molds, (iv) the photo resist was developed followed by rinsing with isopropanol and distilled water. In order to make the flow and control layer, (v) PDMS was molded on a glass substrate for the flow layer, (vi) while PDMS was spin-coated on a glass substrate for the control layer. Both layers were baked for 2 h at 60 °C. The final device was fabricated by (vii) peeling off the flow layer from the master mold and inhibiting the channel barrier by an oil drop from bonding with the membrane of the control layer. (viii) The flow layer was carefully aligned and plasma bonded on the control layer. (ix) Then, the glass substrate was removed from the control layer and the hydrogel particles incorporated in the cage of posts. (x) Finally, the device was sealed by plasma bonding the control layer on a glass substrate.

**Figure 6.8:** The fabrication process for the CFMT. **Making master mold:** (i) Lamination of the dry film resist on the glass substrate. (ii) UV radiation through a photomask. (iii) Baking the master molds. (iv) Development and rinsing followed by a hard baking. **Making flow and control layer:** (v) Moulding PDMS on flow mold. (vi) Spinning PDMS on control mold. **Making final device:** (vii) Inhibition of the channel barrier by an oil drop. (viii) Alignment and plasma bonding of the flow layer on the control layer. (ix) Removing of the glass substrate and incorporation of the hydrogel particle (x) Plasma bonding of the device on a glass substrate.

Because net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene has an equal temperature and pH response, the same grafting density was selected for the experiments with the CFMT. In the first step, rectangular-shaped hydrogel particles were
synthesized via photopolymerization. Figure 6.9 presents the set-up to prepare photo-lithographically patterned hydrogel particles. In order to receive highly uniform-shaped hydrogel particles, the synthesis was conducted under a nitrogen atmosphere with rigorous exclusion of air using a glovebox and degassed precursor solution by purging with argon for 30 min. As shown, the reaction chamber is filled with a precursor solution, closed with a glass substrate and then sandwiched between a PET plate and a photomask. After UV radiation through the photomask, the patterned particles were rinsed with distilled water and manually incorporated in the control channel. Hydrogel particles of different sizes were fabricated, including $900 \times 700 \times 100$ and $700 \times 500 \times 100 \mu m$ (length $\times$ width $\times$ height).

**Figure 6.9:** Set-up to prepare photo-lithographically rectangular-shaped hydrogel particles (PET = polyethylene terephthalate).\[^{[44,184]}\]

Figure 6.10 shows the set-up for the flow rate studies. A pressure pump connected to the inlet of the flow channel applied the pressure and a flow sensor connected to the outlet of the flow channel detected the flow rate. Moreover, a syringe pump provided the control channel with a solution in which the hydrogel could swell or collapse (flow rate of $10 \mu l/min$). In order to assume an equilibrium swelling degree, the hydrogel particle was conditioned for 10 h in the solution of interest before conducting a flow rate study.

Unfortunately, the water uptake of the large hydrogel particle ($900 \times 700 \times 100 \mu m$) based on net-PNiPAam-g-PAA\(_{53}\)-styrene containing 0.6 mol-% PAA\(_{53}\)-styrene was too high in pH 9 buffer solution (appendix Figure 9.10). This resulted in swelling of the hydrogel particle between the restricting posts and, subsequently, clogging of the control channel. Because the control channel was continuously provided with a fresh solution by a syringe pump, the pressure within the control channel constantly increased, which led to partial fragmentation of the hydrogel particle into several pieces. Naturally, the fragmented
Figure 6.10: Set-up for the flow rate studies. A pressure pump applied a pressure at the inlet of the flow channel, a flow sensor detected the flow rate at the outlet of the flow channel, and a syringe pump provided the control channel with the solution in which the hydrogel collapses or swells (flow rate: 10 μl/min). The red arrows denote the direction of the fluid flow in flow channel, whereas the purple arrows denote the direction of fluid flow in control channel.

hydrogel particle was unable to put enough pressure on the membrane to keep the valve closed. Figure 6.11 displayed several pieces of a fragmented hydrogel particle based on net-PNiPAAm-g-PAA_{53}-styrene containing 0.6 mol-% PAA_{53}-styrene after several flow rate experiments. Because attempts to use a smaller hydrogel particle (700 × 500 × 100 μm) were unsuccessful, a lower grafting density of 0.25 mol-% PAA_{53}-styrene was used to reduce the water uptake in pH 9 buffer solution. Figure 6.11 shows a hydrogel particle (900 × 700 × 100 μm) based on net-PNiPAAm-g-PAA_{53}-styrene containing 0.25 mol-% in

Figure 6.11: Schematic of the CFMT (all dimensions in μm) and optical microscope images of the CFMT after several opening and closing experiments using net-PNiPAAm-g-PAA_{53}-styrene containing 0.25 mol-% PAA_{53}-styrene or net-PNiPAAm-g-PAA_{53}-styrene containing 0.6 mol-% PAA_{53}-styrene.[44]
a CFMT after several flow rate studies with an unchanged rectangular-shape without fragmentation.

Figure 6.12 displays the flow rate in the flow channel when the applied pressure gradually increases from 0 to 400 mbar in steps of 10 mbar and, then, reduces back to the initial value in the same way. In order to investigate hysteresis behavior, the gradual pressure change was repeated over three consecutive cycles. The experiment was conducted with a swollen \( \text{net-PNiPAAm-g-PAA}_{53}\)-styrene (0.25 mol-% PAA\(_{53}\)-styrene) hydrogel particle in the control channel using a pH 9 buffer solution (particle size 900 × 700 × 100 μm). As shown, a constant flow rate close to 0 μl/min was detected over the applied pressure range, indicating no fluid flow and emphasizing a tightly closed valve due to the pressure of the hydrogel particle, which kept the membrane nicely in place.

![Graph showing flow rate and pressure over time with pH 9 buffer solution](image)

**Figure 6.12**: Flow rate in the flow channel with a swollen net-PNiPAAm-g-PAA\(_{53}\)-styrene hydrogel containing 0.25 mol-% PAA\(_{53}\)-styrene in the control channel when the applied pressure gradually increases from 0 to 400 mbar in steps of 10 mbar and, then, reduces back to the initial value. The flow channel is tightly closed at the barrier and no fluid flow is detected at the outflow.

As seen in Figure 6.13, the same experiment was conducted with a collapsed net-PNiPAAm-g-PAA\(_{53}\)-styrene (0.25 mol-% PAA\(_{53}\)-styrene) hydrogel particle in the control channel. In order to collapse the hydrogel particle, the control channel was provided with pH 3 buffer solution, pH 9 buffer solution at 40 °C, 1 mol/L NaCl solution, or 40 vol-% EtOH solution, respectively. For the pH, salt, and solvent stimuli, the CFMT opens at a
pressure of around 30 mbar and the flow rate increases then linearly with applied pressure. When the maximum pressure of 400 mbar is reached, the flow rate has consequently the

![Collapsed hydrogel by T, pH, salt, or solvent stimulus](image1)

![Deflected membrane](image2)

Figure 6.13: Flow rate in the flow channel with a collapsed net-PNiPAAm-g-PAA\textsubscript{53}-styrene hydrogel containing 0.25 mol-% PAA\textsubscript{53}-styrene in the control channel when the applied pressure gradually increases from 0 to 400 mbar in steps of 10 mbar and, then, reduces back to the initial value. In total, 4 different stimuli were used to contract the hydrogel particle in the control channel.
highest recorded value with \(~ 100 \mu l/min\). Naturally, when the applied pressure is reduced back to 0 mbar, the flow rate follows in the same way and decreases linearly. This function is reproducible for 3 consecutive cycles. Note that the peak at 80 mbar applied pressure in the first cycle for pH 3 buffer solution is caused by a measuring error (denoted with * in Figure 6.13). A slightly different result was obtained for the temperature response. In this case, the CFMT opens delayed in all three cycles at an applied pressure of around 170 mbar and shows then linearity between applied pressure and flow rate when the pressure is reduced again (delayed open denoted with ¥ in Figure 6.13). This result is attributable to small air bubbles, which block the flow channel at the channel barrier and which occurs when the fluidic chip is warmed. It should be mentioned that a permanent warming of the fluidic chip is necessary to prevent cooling in the control channel due to the fast heat

![Diagram](image.png)

**Figure 6.14:** Flow rate in the flow channel when the applied pressure alternates between 0 and 300 mbar with a swollen hydrogel (left, pH 9 buffer solution) and with a collapsed hydrogel (right, pH 3 buffer solution) in the control channel.
transfer. In order to reduce this effect, the fluidic chip was warmed to just 40 °C instead of the common 50 °C. The set-up can be additionally improved by a thermoelectric element exclusively attached at the area located to the hydrogel.

An important aspect in microfluidics, particularly for an industrial application, are multiple opening-closing cycles. This requires mechanical stability of the grafted hydrogel to withstand the mechanical stress by the deflecting membrane. For this reason, multiple opening and closing cycles were conducted by switching the applied pressure in the flow channel between 0 and 300 mbar. Naturally, the CFMT should remain closed without fluid flow in the flow channel at 0 and 300 mbar with a swollen hydrogel particle in the control channel, while the CFMT should open at 300 mbar and close at 0 mbar with a collapsed hydrogel in the control channel. Figure 6.14 displays multiple switching between 0 and 300 mbar applied pressure in the flow channel with a swollen hydrogel (pH 9 buffer solution) and with a collapsed hydrogel (pH 3 buffer solution) in the control channel (particle size 900 × 700 × 100 μm). As demanded, no fluid flow over 90 consecutive opening-closing cycles was detected when the hydrogel is swollen in pH 9 buffer solution. This indicates permanent resistance without damage of the hydrogel particle. In contrast, when the hydrogel is collapsed in pH 3 buffer solution, the flow rate alternates for 90 opening and closing cycles in the same way as the applied pressure. Importantly, the flow rate consistently switches between ~0 and ~75 μl/min without fluctuations emphasizing usability for a microfluidic application.

6.4 Chapter Summary

In this chapter, grafted net-PNiPAAm-g-PAA53-styrene hydrogels were tested for flow control in two fluidic set-ups. According to literature, a straightforward fluidic test station was developed consisting of a fluid reservoir, an inlet channel, an actuator chamber, and an outlet channel (Figure 6.15).\textsuperscript{[116,171]} Because the actuator chamber is filled with crushed hydrogel particles, the active resistance of the hydrogel particles in the actuator chamber (i.e. swelling degree) directs the flow rate and allows flow control by the local environmental conditions. In order to provide an equal response to temperature and pH, the size change of disk-shaped net-PNiPAAm-g-PAA53-styrene samples after applying temperature or pH stimulus was evaluated. Size change studies indicated that a PAA53-styrene content of 0.6 mol-% results in an equal size change for the temperature and pH stimulus.

The flow rate experiments using the straightforward test station were conducted with two cylinder-shaped actuator chambers of different sizes (10 × 4 and 10 × 1 mm). In case that an actuator chamber with a chamber size of 10 × 4 mm was used, the fluid flow
shut off when the inlet channel was provided with a pH 9 buffer solution at r.t., while it immediately opened when a pH 9 buffer solution at 50 °C was used. However, the chamber size of $10 \times 4$ mm had the disadvantage of considerably slower opening and closing times when the pH, salt, and solvent stimulus was employed. This drawback could be overcome by an actuator chamber with a smaller chamber size of $10 \times 1$ mm. Using this actuator chamber, accelerated opening and closing function over six consecutive cycles was achieved applying pH, temperature, and salt stimuli. In contrast, unreproducible flow rates were obtained with the solvent stimuli, which was contributed to air bubbles as a result of the side reaction of ethanol and the pH 9 buffer solution. The advantageous properties of net-PNiPAAm-g-PAA$_{53}$-styrene were highlighted by using pH, salt, and solvent stimulus

**Figure 6.15:** Operating principle of (top) the straightforward fluidic test station with an actuator chamber filled with crushed hydrogel particles (net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene) and (bottom) the CFMT with a thin membrane separating hydrogel particle and flow channel (net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene).
in one experiment. Remarkably, the opening and closing function was reversible for six consecutive cycles.

As part of a collaboration project with the chair of polymeric microsystems within the Cluster of Excellence Center for Advancing Electronics Dresden (A. Richter and P. Frank), grafted net-PNiPAAm-g-PAA$_{53}$-styrene hydrogels were tested in a microfluidic chip in which hydrogel particle and flow channel are separated by a thin flexible membrane (Figure 6.15). Because water uptake of net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene was too high accompanied by clogging of the control channel, the grafting density was reduced to 0.25 mol-% PAA$_{53}$-styrene. As demanded, when a pressure was applied in the flow channel and the hydrogel was swollen in pH 9 buffer solution, the CFMT remained tightly closed allowing no fluid flow in the flow channel. On the contrary, when a pressure was applied in the flow channel and the hydrogel was collapsed by one of the four stimuli (i.e. temperature, pH, salt, or solvent), the CFMT opened because of the deflection of the membrane downward. In these cases, the flow rate linearly increased with applied pressure in the flow channel. Multiple opening and closing cycles showed no considerable changes in the CFMT function emphasizing a high potential for an application in microfluidics.
7 Conclusion and Future Prospects

Conclusion

In this work, a temperature- and pH-responsive hydrogel for the application as a chemomechanical valve in microfluidics was developed. As the building blocks, the well-known temperature- and pH-responsive monomers NiPAAm and AA were selected. Because it is known that long NiPAAm segments are needed to retain the thermoresponsive behavior of PNiPAAm, the approach of grafted hydrogels with a PNiPAAm backbone and PAA graft chains was chosen. As shown in Figure 7.1, a series of pH-responsive macromonomers was prepared via RAFT polymerization. Four different end-group functionalizations of the macromonomer were evaluated, including allyl, vinyl, acrylamide, and styrene.

The functionalization with an allyl end-group was unsuitable for this work due to the low degree of end-group functionalization. Macromonomers with a unconjugated vinyl end-group were left out as well because the unconjugated vinyl end-group was not incorporated into PNiPAAm. Having failed to use allyl and unconjugated vinyl end-groups as functionalization, two alternative approaches based on CuAAC were developed. According to the first approach, azide-bearing polymers were prepared and functionalized via CuAAC to obtain an acrylamide-functionalized macromonomer. However, ATR-IR analysis indicated that exclusively electron-rich monomers, such as VBA and 4VP, are suitable for this approach due to the side reaction of electron-poor monomers (e.g. AA) with the azide end-group. In the second approach, alkyne-functionalized polymers were synthesized via RAFT polymerization followed by the introduction of the styrene end-group using CuAAC as well. Importantly, this approach was suitable for electron-rich and electron-poor monomers.

A series of grafted net-PNiPAAm-g-PVBA26-AAm hydrogels with a grafting density between 0 and 1 mol-% PVBA26-AAm content was prepared. The grafting efficiency was calculated to be above 70 % indicating good incorporation of the PVBA26-AAm graft chains into the PNiPAAm backbone. Equilibrium swelling studies displayed that a PVBA26-AAm content of 0.5 mol-% is sufficient to achieve an adequate swelling response to temperature and pH (ratio Q_{m,i} > 3). Remarkably, the temperature transition retained sharp and was mainly unaffected by the incorporated PVBA26-AAm graft chains. Furthermore,
Conclusion and Future Prospects

Salt response studies showed that \textit{net-PNiPAAm-g-PVBA}_{26}-AAm containing 0.5 mol-% PVBA\textsubscript{26}-AAm exhibits a less pronounced salt response compared to pure \textit{net-PNiPAAm}, which was contributed to stabilized hydrogen-bonding by the PVBA\textsubscript{26}-AAm graft chains. In addition, the cononsolvency behavior of \textit{net-PNiPAAm-g-PVBA}_{26}-AAm containing 0.5 mol-% PVBA\textsubscript{26}-AAm was analyzed by equilibrium swelling experiments using methanol, ethanol, 1-propanol, and acetone. High solvent response with ratio \(Q_{m,\text{solvent}}\) values between 20 and 100 were detected, which is suitable for an application as a stimulus in microfluidics.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{functionalizations.png}
\caption{Four different end-group functionalizations for the synthesis of macromonomers were investigated: (i) an allyl-functionalization, (ii) a unconjugated vinyl-functionalization, (iii) an acrylamide-functionalization, and (iv) a styrene functionalization.}
\end{figure}

An important aspect of a hydrogel for flow control is a reversible swelling-deswelling behavior. When the swelling agent was alternated between pH 9 and pH 3 buffer solution,
a reversible swelling-deswelling behavior of net-PNiPAAm-g-PVBA26-AAm over repeated cycles was observed (Figure 7.2). However, a less pronounced pH response was detected after the first cycle possibly due to ionic hydrations of the buffer solution by the PVBA26-AAm graft chains associated with a decreased osmotic pressure. This phenomenon was observed at r.t. as well as at 50 °C. Moreover, a reversible swelling-deswelling behavior of net-PNiPAAm-g-PVBA26-AAm was observed in pH 9 buffer solution when the temperature of the swelling agent was changed from r.t. to 50 °C, and vice versa. On the contrary, when the experiment was conducted with a pH 3 buffer solution as the swelling agent, a poor swelling-deswelling behavior was noticed. The phenomenon was attributed to intrachain complexes due to π-interactions and hydrogen bonds between amide groups and protonated carboxylic groups as well as between carboxylic groups of single graft chains. These intrachain complexes prevent the water uptake of grafted net-PNiPAAm-g-PVBA26-AAm hydrogels when the temperature of the swelling agent was changed from 50 °C to room temperature.

The mechanical properties of grafted net-PNiPAAm-g-PVBA26-AAm hydrogels were evaluated by rheological analysis. Unfortunately, due to the high water uptake, grafted net-PNiPAAm-g-PVBA26-AAm hydrogels with a PVBA26-AAm content above 0.25 mol-% revealed extremely poor mechanical properties unsuitable for microfluidic systems with any mechanical stress. For this reason, net-PNiPAAm-g-PVBA26-AAm was not taken into account for the following flow studies.

Figure 7.2: Optical microscope images of (left) net-PNiPAAm-g-PVBA26-AAm containing 1 mol-% PVBA26-AAm and (right) net-PNiPAAm-g-PAA53-styrene containing 0.5 mol-% PAA53-styrene at different conditions: (pH 9, r.t.) hydrogel totally swollen; (pH 9, r.t. and pH 3, 50 °C) hydrogel partially collapsed; (pH 3, 50 °C) hydrogel completely collapsed.
Grafted net-PNiPAAm-g-PAA₅₃-styrene hydrogels with a PNiPAAm backbone and PAA₅₃-styrene graft chains of different molecular weights were prepared. Equilibrium swelling studies of the pH stimulus indicated that net-PNiPAAm-g-PAA₅₃-styrene containing PAA₅₃-styrene graft chains with a molecular weight of 4200 g/mol reveals a good balance between pH response and potentially resulting mechanical stability. A series of grafted net-PNiPAAm-g-PAA₅₃-styrene hydrogels were prepared and studied in terms of grafting efficiency, response behavior, mechanical properties, response time, and photopolymerization. The grafting efficiency was calculated to be above 90 % implying quantitative incorporation of PAA₅₃-styrene into the PNiPAAm backbone. Equilibrium swelling studies displayed that a grafting density between 0.25 and 1 mol-% is sufficient to provide an adequate swelling response to temperature and pH stimuli (ratio Q_m,i > 3). In addition, equilibrium swelling studies employing NaCl and water-ethanol mixtures showed that salt and solvent response are suitable stimuli for an application in this study. However, the temperature transition of net-PNiPAAm-g-PAA₅₃-styrene was broader compared to net-PNiPAAm-g-PVBA₂₆-AAm and the VPTT was shifted to higher temperatures. Because an application as a chemo-mechanical valve with a reproducible opening and closing function was aimed, the swelling-deswelling behavior of net-PNiPAAm-g-PAA₅₃-styrene was evaluated, which turned out to be reversible over 5 repeated cycles. Unlike net-PNiPAAm-g-PVBA₂₆-AAm, a reversible swelling-deswelling behavior in pH 3 buffer solution was observed when the temperature of the swelling agent was alternated between r.t. and 50 °C (Figure 7.2).

Table 7.1: Grafting efficiency, VPTT, corr. D_coop, response time, storage modulus, and gelation time for net-PNiPAAm-g-PAA₅₃-styrene hydrogels with different grafting densities.

<table>
<thead>
<tr>
<th>PAA₅₃-styrene efficiency</th>
<th>VPTT</th>
<th>corr. D_coop</th>
<th>Response</th>
<th>Storage modulus</th>
<th>Gelation time</th>
</tr>
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<tr>
<td>[mol-%]</td>
<td>[%]</td>
<td>[°C]</td>
<td>[cm²/s]</td>
<td>[s]</td>
<td>[kPa]</td>
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<tr>
<td>0</td>
<td>-</td>
<td>34.9 ± 0.1</td>
<td>4.4 ± 0.7·10⁻⁷</td>
<td>23.8 ± 2.7</td>
<td>37.2 ± 1.3</td>
</tr>
<tr>
<td>0.25</td>
<td>91</td>
<td>36.4 ± 0.1</td>
<td>1.6 ± 0.3·10⁻⁶</td>
<td>6.5 ± 1.3</td>
<td>19.7 ± 0.9</td>
</tr>
<tr>
<td>0.5</td>
<td>98</td>
<td>41.3 ± 0.2</td>
<td>2.3 ± 0.4·10⁻⁶</td>
<td>4.4 ± 0.9</td>
<td>10.1 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>94</td>
<td>43.0 ± 2.2</td>
<td>4.0 ± 0.2·10⁻⁶</td>
<td>2.6 ± 0.1</td>
<td>9.5 ± 0.3</td>
</tr>
</tbody>
</table>

* Properties of net-PNiPAAm-g-PAA₅₃-styrene prepared via FRP as described in chapter 5.2. ^b^ Determined in water by UV/Vis using the cloud point method.[156] ^c^ Gelation time in a photopolymerization as described in chapter 5.7.

The collective diffusion model extended by a volume specific surface was applied to determine the rate of the volume phase transition.[125] Swelling kinetic studies revealed that
the cooperative diffusion coefficient of net-PNiPAAm-g-PAA\textsubscript{53}-styrene increases with the grafting density of PAA\textsubscript{53}-styrene. Net-PNiPAAm-g-PAA\textsubscript{53}-styrene containing 1 mol-% PAA\textsubscript{53}-styrene revealed an accelerated rate of volume phase transition by a factor of 9 compared to pure net-PNiPAAm. This is particularly beneficial for the application as a chemo-mechanical valve. Rheological analysis of net-PNiPAAm-g-PAA\textsubscript{53}-styrene showed that an increasing graft density leads to decreasing mechanical stability. Even so, net-PNiPAAm-g-PAA\textsubscript{53}-styrene revealed higher mechanical strength compared to net-PNiPAAm-g-PVBA\textsubscript{26}-AAm. The photopolymerization experiments showed that the gelation time linearly increases with the grafting density. Table 7.1 summarizes the properties of net-PNiPAAm-g-PAA\textsubscript{53}-styrene.

In the next step, grafted net-PNiPAAm-g-PAA\textsubscript{53}-styrene hydrogels were tested as a chemo-mechanical valve in two fluidic set-ups. Because an equal response (i.e. volume change) independent from the applied stimulus is demanded in many applications in microfluidics, the size change of disk-shaped net-PNiPAAm-g-PAA\textsubscript{53}-styrene samples with different grafting densities after applying temperature or pH stimulus was determined. Size change studies indicated that a PAA\textsubscript{53}-styrene content of 0.6 mol-% results in an equal size change for the temperature and pH stimulus.

A straightforward fluidic test station consisting of a fluid reservoir, an inlet channel, an actuator chamber, and an outlet channel was constructed (Figure 7.3). The actuator chamber was filled with crushed hydrogel particles of net-PNiPAAm-g-PAA\textsubscript{53}-styrene containing 0.6 mol-% PAA\textsubscript{53}-styrene and allowed to regulate the fluid flow by hydrogel volume in the actuator chamber (i.e. swelling degree). Two actuator chambers of different sizes were used for the flow rate studies (10 × 4 and 10 × 1 mm). In the case of a fluidic test station equipped with the actuator chamber of 10 × 4 mm, the actuator chamber shut off the fluid flow when the inlet channel was provided with a pH 9 buffer solution at r.t., while a fluid flow was detected when a pH 9 buffer solution at 50 °C was used. Unfortunately, the actuator chamber opened considerably delayed when the pH, salt, and solvent stimulus were employed.

Flow rate studies of the fluidic test station equipped with the actuator chamber of 10 × 1 mm showed that the flow rate was significantly throttled when the pH 9 buffer solution at r.t. was used. In contrast, when the inlet was provided with a solution in which the hydrogel collapses (pH 3 buffer solution, pH 9 buffer solution at 50 °C, 40 vol-% EtOH solution, or 1 mol/L NaCl solution), the flow rate immediately increases about ~ 2 - 2.5 times. In order to highlight the properties of net-PNiPAAm-g-PAA\textsubscript{53}-styrene, pH value, salt, and solvent stimulus were employed in one experiment. Remarkably, the opening and closing function of the actuator chamber was reversible over six consecutive cycles (Figure 7.3).
Figure 7.3: (right) Operating principle of the straightforward fluidic test station with an actuator chamber filled with crushed hydrogel particles (net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene). The flow can be controlled by 4 different stimuli. (left) Corrected flow rate plotted as a function of the outflow. The solution was changed between pH 9 buffer solution at r.t. and one solution in which the hydrogel collapses. The solution in which the hydrogel collapses was alternated between pH 9 buffer solution at 50 °C, pH 3 buffer solution, and 1 mol/L NaCl solution. The color background of the diagram represents the provided solution. An actuator chamber with a size of 10 × 1 mm filled with crushed particles of net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene was used in this experiment.\cite{43}

Grafted net-PNiPAAm-g-PAA$_{53}$-styrene hydrogels were tested in a microfluidic chip, more specifically a chemo-fluidic membrane transistor, in which hydrogel particle and flow channel are separated by a thin flexible membrane (Figure 7.4). For the CFMT, the grafting density of net-PNiPAAm-g-PAA$_{53}$-styrene was reduced to 0.25 mol-% PAA$_{53}$-styrene due to the high water uptake of net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.6 mol-% PAA$_{53}$-styrene associated with clogging of the control channel. Flow rate studies with CFMT and net-PNiPAAm-g-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene showed that the CFMT remained tightly closed allowing no fluid flow in the flow channel when a pressure was applied in the flow channel and the hydrogel was swollen (the control channel was provided with a pH 9 buffer solution). In contrast, the CFMT opened due to the deflection of the membrane when a pressure was applied in the flow channel and the hydrogel was collapsed by one of the four stimuli (the control channel was provided with a pH 9 buffer solution at 50 °C, a pH 3 buffer solution, 40 mol-% EtOH solution, or 1 mol/L NaCl solution, respectively). Importantly, there was a linear relationship between applied pressure and resulting flow rate in the flow channel. Multiple opening and closing switches
over 90 cycles without considerable changes in the flow rate emphasized a high industrial potential.

**Figure 7.4:** Operating principle of the chemo-fluidic membrane transistor in which hydrogel particle and flow channel are separated by a thin flexible membrane. Four different stimuli can be applied when *net*-PNiPAAm-9-PAA$_{53}$-styrene containing 0.25 mol-% PAA$_{53}$-styrene is incorporated in the control channel.

### Future Prospects

The presented material was tailored to be responsive to four individual stimuli with high volume change. Unfortunately, rheological analysis indicated decreasing mechanical stability with increasing grafting density due to the high water uptake. A good starting point for future works is to improve the mechanical properties of the hydrogel. It is possible that increasing the cross-linking density could lead to improved mechanical properties. Consequently, the influence of the cross-linking density on the mechanical stability should be investigated. Alternatively, automatic reparation of damages due to self-healing properties may extend the lifetime. Self-healing is typically achieved by dynamic and reversible cross-links. Figure 7.5 presents a promising approach based on host-guest interactions with adamantane and cyclodextrin. Adamantane and cyclodextrin molecules with polymerizable vinyl groups would need to be prepared. Another way to reach physical cross-links would be to use complementary H-bonding acceptor and donor molecules like the ureidopyrimidinone unit. However, a higher cross-linking density may decrease the responsiveness towards environmental changes and should be validated.

As shown, the cooperative diffusion coefficient decreases with PAA$_{53}$-styrene content, which indicated faster response times with increasing grafting density. However, all $D_{\text{coop}}$
values were exclusively determined with the temperature stimulus. The \( D_{\text{coop}} \) values using pH, salt, and solvent stimuli would be worthy of investigation, particularly to compare these three stimuli with the temperature stimulus. Furthermore, the cooperative diffusion coefficient should be determined by dynamic light scattering, which could additionally confirm the data obtained.

As shown in chapter 5.7, \textit{net-PNiPAAm-g-PAA}_{53}-styrene hydrogels can be obtained via photopolymerization. It would be interesting to investigate key properties of \textit{net-PNiPAAm-g-PAA}_{53}-styrene prepared with this method (e.g. response studies, diffusion coefficient, mechanical strength).

Other interesting parameters, which should be evaluated are the compression modulus and the degree of compression at break. These values give further information about the prepared materials and make the relationship between macromonomer content and mechanical properties clearer.

Because the response to temperature and pH stimulus can be controlled with the grafting density, it expands potential applications of tetra-responsive grafted hydrogels to other interesting research fields like drug delivery, tissue engineering, and catalysis. Furthermore, tetra-responsive grafted hydrogels could be useful materials in microfluidics for chemostats and micropumps.
Experimental
8 Methods, Instruments, and Materials

Materials

Regenerated Celluloses tubings membranes with MWCO 1000 were received from Carl Roth. Sintered filter disks were received from ROBU with porosity 0 and 10 mm diameter. Glass substrates (Borofloat33) were received from Schott Jena.

NMR Spectroscopy

The NMR experiments were performed on a Bruker Avance III 500 NMR spectrometer operating at 500.13 MHz for $^1$H NMR and at 125.75 MHz for $^{13}$C NMR. CD$_3$Cl, DMSO-$d_6$, or D$_2$O were used as the solvents. $^1$H and $^{13}$C NMR spectra are referenced to the employed solvent. The following abbreviations are used for the multiplicities: s - singulet, d - doublet, t - triblet, q - quadruplet, m - multiplet, br - broad.

IR and Raman Spectroscopy

The IR experiments were performed on a Bruker Tensor 27 system equipped with a mid-IR source (4000 to 500 cm$^{-1}$), diamond-ATR unit, and KBr beamsplitter. Samples were analyzed as a dry film by drop casting from EtOH or as a powder. Acquired IR spectra were interpreted with Bruker OPUS Data Collection and Analysis FT-IR Software Program.

Raman spectra were collected on a RAMAN Imaging System WITEC alpha300R equipped with a pulsed 532 nm Nd: YAG laser (laser power: 5 - 10 mW, zoom: 20×, integration time: 0.5 - 1 s, accumulation: 200 - 500). Samples were measured as a powder in a standard accessory.

Differential Scanning Calorimetry

The DSC experiments were performed on a DSC Q 2000 TA Instruments.
UV/Vis Spectroscopy

The UV/Vis experiments were performed on a Varian Cary 50 UV-Vis equipped with a xenon flash lamp and a thermostatted sample holder.

MALDI-TOF MS

The MALDI-TOF experiments were performed on a Bruker autoflex speed TOF/TOF equipped with Nd: YAG laser. The samples were analyzed with THF as solvent and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile for P4VP\textsubscript{38}-AAm or dithranol for PVBA\textsubscript{26}-AAm as the matrix.

Size Exclusion Chromatography

GPC analysis of PAA\textsubscript{n}-alkyne was performed on an Agilent 1200 Series equipped with a 1 PL aquagel-OH MIXED-H (8 µm, 300 × 7.5 mm ID) column and a K2301 detector (solvent: 0.01 M NaH\textsubscript{2}PO\textsubscript{4}·H\textsubscript{2}O + 0.5 M NaCl, flow rate: 1 ml/min, standard: PAA). GPC analysis of PVBA\textsubscript{26}-N\textsubscript{3} was performed on a PL-GPC 50 Plus equipped with a refractive index detector and a ResiPore column from Agilent (solvent: THF, flow rate: 1 ml/min, standard: PS). PVBA\textsubscript{26}-N\textsubscript{3} was converted to azide-functionalized poly(methyl-4-vinylbenzoate) via methylation using MeOH, DCC, and DMAP before conducting the SEC measurement. GPC analysis of P4VP\textsubscript{38}-N\textsubscript{3} was performed on an Agilent 1200 Series equipped with two ZORBAX PSM columns and an RI-D 1000 detector (solvent: DMAc + 2 vol-% H\textsubscript{2}O + 3 g/L LiCl, flow rate: 1 ml/min, standard: P2VP).

UV Lamp

Samples were irradiated by an EXFO OmniCure 1000 spot curing system equipped with a high pressure mercury lamp (100 W Mercury Vapor short Arc and 320 - 500 nm filter).

Rheometer

Rheology analysis was performed on an ARES-G2 rheometer with a parallel plates geometry. Acquired spectra were interpreted with TA Instrument TRIOS software.

Optical Microscope

All optical microscope experiments were performed on a LEICA S8 APO. Acquired images were analyzed with Fiji Image J software program.
**Plasma Oven**

All oxygen plasma activation were performed on a DREVA Clean 450 (Vakuumtechnik Dresden GmbH, Germany).

**Flow Sensor**

All flow rates described in chapter 6.3 were measured with an SLI-1000 (Sensirion, Switzerland).

**Chemicals**

Unless otherwise specified, all chemicals were of analytical grade and used as received without further purification. N-Isopropylacrylamide was purified by recrystallization from hexane, and vacuum dried. Acrylic acid was purified by distillation under vacuum and stored under inert gas.

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9 Synthetic Procedures

9.1 Allyl-Functionalization

General Procedure for the Preparation of Allyl-Functionalized DTP, as Exemplified for the Synthesis of DTP-allyl amide

DTP (367.1 mg, 1.01 mmol) and PyBOP (654.9 mg, 1.26 mmol) were dissolved in 5 ml of CH$_2$Cl$_2$ under argon atmosphere. After being stirred at room temperature for 2 h, 2-propen-1-ylamine (57.1 mg, 1 mmol) and DIPEA (164.4 mg, 1.27 mmol) in 5 ml of CH$_2$Cl$_2$ was added, and the reaction mixture was stirred for 3 d. The solvent was evaporated under reduced pressure and the raw product was purified by column chromatography using 0 - 10 % ethyl acetate in n-hexane (best separation effect at 6 %) resulting in a yellow solid.

**Yield:** 361 mg (0.90 mmol, 90 %)

**R$_f$ value:** 0.33 (SiO$_2$ / 20 % ethyl acetate)

$^1$H NMR (500 MHz, CDCl$_3$)

$\delta$ [ppm] = 0.89 (t, $J = 6.9$ Hz, 1H, 1-H), 1.27 - 1.41 (m, 18H, 2-H to 10-H), 1.66 - 1.72 (m, 8H, 11-H and 15-H), 3.29 (t, $J = 7.3$ Hz, 2H, 12-H), 3.86 (tt, $J = 5.7$ Hz, 1.5 Hz, 2H, 18-H), 5.14 (m, 2H, 20-H), 5.8 (m, 1H, 19-H), 6.55 (br. s., 1H, 17-H).

$^{13}$C NMR (126 MHz, CDCl$_3$)

$\delta$ [ppm] = 14.08 (CH$_3$, 1-C), 22.66 (CH$_2$, 2-C), 25.92 (CH$_3$, 15-C), 27.75 (CH$_2$, 11-C), 28.91 - 29.59 (7 × CH$_2$, 4-C to 10-C), 31.89 (CH$_2$, 3-C), 37.08 (CH$_2$, 12-C), 42.74 (CH$_2$, 18-C), 57.19 (C$_q$, 14-C), 116.52 (CH$_2$, 20-C), 134.06 (CH, 19-C), 172.26 (C$_q$, 16-C), 220.01 (C$_q$, 13-C).
$^1$H NMR (500 MHz, CDCl$_3$)  
$\delta$ [ppm] = 0.89 (t, $J = 6.9$ Hz, 1H, 1-H), 1.27 - 1.33 (m, 16H, 2-H to 9-H), 1.39 (m, 2H, 9-H), 1.67 (m, 2H, 11-H), 1.72 (m, 6H, 15-H), 3.28 (t, $J = 7.5$ Hz, 2H, 12-H), 4.61 (dt, $J = 5.8$ Hz, 1.4 Hz, 2H, 17-H), 5.21 - 5.35 (m, 2H, 19-H), 5.91 (m, 1H, 18-H).

$^{13}$C NMR (126 MHz, CDCl$_3$)  
$\delta$ [ppm] = 14.08 (CH$_3$, 1-C), 22.66 (CH$_2$, 2-C), 25.92 (CH$_3$, 15-C), 27.75 (CH$_2$, 11-C), 28.91 - 29.59 (7 $\times$ CH$_2$, 4-C to 10-C), 31.89 (CH$_2$, 3-C), 37.08 (CH$_2$, 12-C), 42.74 (CH$_2$, 18-C), 57.19 (C$_q$, 14-C), 116.52 (CH$_2$, 20-C), 134.06 (CH, 19-C), 172.26 (C$_q$, 16-C), 220.01 (C$_q$, 13-C).

**General Procedure for the Polymerization Using the Allyl-Functionalized DTP, as Exemplified for the Synthesis of PAA$_{72}$-allyl amide**

DTP-allyl amide (50 mg, 0.124 mmol), AA (1.4 g, 19.43 mmol) and ACP (2.4 mg, 8.56 µmol) were dissolved in 6.2 ml of N,N-dimethylformamide under argon atmosphere. The solution was degassed by several cycles of the freeze-pump-thaw procedure and then stirred at 70 °C for 6 h. The reaction mixture was exposed to air, quenched with liquid nitrogen and precipitated in CHCl$_3$ from THF. After collecting the yellow precipitate by filtration, the product was dried under vacuum at 40 °C.

In order to investigate the monomer conversion by $^1$H NMR spectroscopy, 0.25 ml of the reaction mixture were taken at 0, 0.5, 1, 2, 3, 4 and 6 h, exposed to air, and quenched with liquid nitrogen.

**Yield:** 50 % (determined with $^1$H NMR)

**$M_n$,NMR:** 5600 g/mol
9.2 Unconjugated Vinyl-Functionalization

Synthesis of DTP-vinyl ester

DTP (369.3 mg, 1.01 mmol) and PyBOP (538.3 mg, 1.03 mmol) were dissolved in 5 ml of CH$_2$Cl$_2$. After being stirred at room temperature for 2 h, 3-buten-1-ol (75.11 mg, 1 mmol) and DIPEA (329.2 mg, 2.56 mmol) in 5 ml of CH$_2$Cl$_2$ was added, and the reaction mixture was stirred for 3 d. The solvent was evaporated under reduced pressure and the raw product was purified by column chromatography using 0 - 10 % ethyl acetate in n-hexane (best separation effect at 4 %) resulting in yellow ochre liquid.

Yield: 271 mg (0.65 mmol, 65 %)

R$_f$ value: 0.66 (SiO$_2$ / 20 % ethyl acetate)

$^1$H NMR (500 MHz, CDCl$_3$)

δ [ppm] = 0.89 (t, $J = 7.0$ Hz, 1H, 1-H), 1.23 - 1.43 (m, 18H, 2-H to 10-H), 1.63 - 1.72 (m, 8H, 11-H and 15-H), 2.39 (m, 2H, 18-H), 3.28 (t, $J = 7.5$ Hz, 12-H), 4.16 (t, $J = 6.6$ Hz, 17-H), 5.08 (m, 2H, 20-H), 5.77 (m, 1H, 19-H).
13C NMR (126 MHz, CDCl₃)
δ [ppm] = 14.06 (CH₃, 1-C), 22.64 (CH₂, 2-C), 25.36 (CH₃, 15-C), 27.84 (CH₂, 11-C), 28.87 - 29.58 (7 × CH₂, 4-C to 10-C), 31.88 (CH₂, 3-C), 36.89 (CH₂, 12-C), 55.93 (Cq, 14-C), 66.64 (CH₂, 17-C), 118.27 (CH₂, 20-C), 131.85 (CH, 19-C), 172.61 (Cq, 16-C), 221.26 (Cq, 13-C).

General Procedure for the Polymerization Using DTP-vinyl ester, as Exemplified for the Synthesis of PAA₄₆-vinyl ester

DTP-vinyl ester (200 mg, 0.423 mmol), AA (1.76 g, 24.41 mmol) and ACP (6.6 mg, 23.5 μmol) were dissolved in 8.2 ml of N,N-dimethylformamide under argon atmosphere. The solution was degassed by several cycles of the freeze-pump-thaw procedure and then stirred at 70 °C for 6 h. The reaction mixture was exposed to air, quenched with liquid nitrogen and precipitated in CHCl₃. After collecting the yellow precipitate by filtration, the product was dried under vacuum at 40 °C.

Yield: 92 % (determined with ¹H NMR)

Mₙ,NMR: 3700 g/mol

Mₙ,SEC: 2000 g/mol (in 0.01 M NaH₂PO₄·H₂O + 0.5 M NaCl, calibration based on PAA standards)

Mₜ/Mₙ: 1.9

¹H NMR (500 MHz, DMSO-­d₆)
δ [ppm] = 0.85 (m, 1-H), 1.2 - 1.9 (m, 2-H to 11-H, 14-H), 2.0 - 2.4 (br. s., 13-H), 3.60 (m, 2H, 12-H), 4.01 (m, 2H, 17-H), 5.07 (m, 20-H), 5.78 (m, 19-H), 11.5 - 13 (br. s., 15-H).
Control Experiment to Evaluate the Grafting Efficiency of PAA$_{46}$-vinyl ester

PAA$_{46}$-vinyl ester (22.9 mg, 0.0062 mmol), NiPAAm (141.5 mg; 1.25 mmol) and BIS (2.89 mg, 0.0188 mmol) were dissolved in 0.8 ml of water (pH 10) under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, potassium persulfate (2.05 mg, 0.0125 mmol) was added, followed by 1 µl of DIPEA. The reaction mixtures transferred into a glass tube of 3 mm diameter, sealed under argon and submerged in a water bath maintained at 10 °C. After the reaction was carried out for 1 d, the gel was separated from the glass tube and washed several times with water.

9.3 Acrylamide-Functionalization

Synthesis of $N$-(prop-2-yn-1-yl)acrylamide

2-Propen-1-ylamine (1.28 ml, 20 mmol) was dissolved in 20 ml of CH$_2$Cl$_2$ under an argon atmosphere and cooled in ice. Acryloyl chloride (2.5 ml, 27 mmol) in 10 ml of CH$_2$Cl$_2$ was slowly added, and the resulting mixture was stirred at r.t. for 3 d. The reaction mixture was diluted with 20 ml of water, the precipitate was removed by filtration, and the solution was extracted with diethyl ether (4 × 40 ml). The organic phase was dried over MgSO$_4$, the solvent was evaporated under reduced pressure, and the raw product was purified by column chromatography using 0 - 40 % ethyl acetate in n-hexane (best separation effect at 40 %) resulting in a white yellow solid.

Yield: 1.5 g (0.65 mmol, 67 %)

$R_f$ value: 0.66 (SiO$_2$ / 20 % ethyl acetate)

$^1$H NMR (500 MHz, CDCl$_3$)

$\delta$ [ppm] = 2.25 (t, $J = 2.8$ Hz, 1H, 1-H), 4.13 (dd, $J = 5.4$ Hz, 2.5 Hz, 2H, 3-H), 5.69 (m, 1H, 7-H), 6.01 (br. s., 1H, 4-H), 6.14 (m, 1H, 6-H), 6.32 (m, 1H, 7-H).

$^{13}$C NMR (126 MHz, CDCl$_3$)
δ [ppm] = 29.18 (CH$_2$, 3-C), 71.58 (CH, 1-C), 79.31 (C$_q$, 2-C), 127.08 (CH$_2$, 7-C), 130.18 (CH, 6-C), 165.28 (C$_q$, 5-C).

### Synthesis of 3-azido-1-propylamine

3-Chloropropylamine hydrochloride (8.2 g, 63.1 mmol) and sodium azide (12.6 g, 193.8 mmol) were dissolved in 50 ml of water and stirred at 80 °C for 24 h under reflux. After the solution was basified with sodium hydroxide, the reaction mixture was extracted with diethyl ether (4 × 40 ml). The organic phase was dried over MgSO$_4$, and the solvent was evaporated under reduced pressure resulting in a volatile, colorless liquid.

**Yield:** 4.1 g (41 mmol, 65 %)

![3-azido-1-propylamine](image)

$^1$H NMR (500 MHz, CDCl$_3$)

δ [ppm] = 1.26 (br. s., 2H, 4-H), 1.74 (quin, $J = 6.8$ Hz, 2H, 2-H), 2.82 (t, $J = 6.8$ Hz, 2H, 3-H), 3.39 (t, $J = 6.7$ Hz, 2H, 1-H).

$^{13}$C NMR (126 MHz, CDCl$_3$)

δ [ppm] = 32.1 (CH$_2$, 2-C), 39.1 (CH$_2$, 3-C), 49.1 (CH$_2$, 1-C).

### Synthesis of DTP-N$_3$

DTP (915 mg, 2.51 mmol) and PyBOP (1470 mg, 2.83 mmol) were dissolved in 8 ml of CH$_2$Cl$_2$. After being stirred at room temperature for 2 h, 3-azido-1-propylamine (250 mg, 2.5 mmol) and DIPEA (797 mg, 6.17 mmol) in 2 ml of CH$_2$Cl$_2$ was added, and the reaction mixture was stirred for 3 d. The solvent was evaporated under reduced pressure and the raw product was purified by column chromatography using 0 - 20 % ethyl acetate in n-hexane (best separation effect at 20 %) resulting in yellow solid.

**Yield:** 1009 mg (2.3 mmol, 90 %)

**R$_f$ value:** 0.66 (SiO$_2$ / 20 % ethyl acetate)
1H NMR (500 MHz, DMSO-\textit{d}_6)
\(\delta [\text{ppm}] = 0.85 (t, J = 7 \text{ Hz}, 3\text{H}, 1\text{-H}), 1.21 - 1.38 (m, 18\text{H}, 2\text{-H to 10\text{-H}}), 1.55 - 1.68 (m, 10\text{H}, 11\text{-H to 12\text{-H}} and 15\text{-H}), 3.1 (q, J = 6.3 \text{ Hz}, 2\text{H}, 18\text{-H}), 3.25 - 3.35 (m, 4\text{H}, 19\text{-H} and 20\text{-H}), 7.97 (t, J = 5.2 \text{ Hz}, 1\text{H}, 17\text{-H}).

13C NMR (126 MHz, DMSO-\textit{d}_6)
\(\delta [\text{ppm}] = 14.07 (\text{CH}_3, 1\text{-C}), 22.65 (\text{CH}_2, 2\text{-C}), 25.85 (\text{CH}_3, 15\text{-C}), 27.74 (\text{CH}_2, 11\text{-C}), 28.92 - 29.60 (8 \times \text{CH}_2, 4\text{-C to 10\text{-C}} and 19\text{-C}), 31.88 (\text{CH}_2, 3\text{-C}), 37.12 (\text{CH}_2, 20\text{-C}), 37.93 (\text{CH}_2, 12\text{-C}), 49.53 (\text{CH}_2, 18\text{-C}), 57.16 (\text{C}_q, 14\text{-C}), 172.64 (\text{C}_q, 16\text{-C}), 220.31 (\text{C}_q, 13\text{-C}).

IR (film, ATR-IR)
\(\tilde{\nu} [\text{cm}^{-1}] = 3353 (\nu_{\text{NH}}), 2914 (\nu_{\text{CH}_2, \text{as}}), 2850 (\nu_{\text{CH}_2, \text{s}}), 2088 (\nu_{\text{N}_3}), 1648 (\text{Amide I}), 1523 (\text{Amide II}), 1469 (\delta_{\text{CH}_2}), 1245 (\text{Amide III}), 1066 (\nu_{\text{C=S}}).

**Control Experiment with DTP-N\textsubscript{3} Using AA or VBA as the Monomer, as Exemplified for AA**

DTP-N\textsubscript{3} (50 mg, 0.11 mmol) and AA (33 mg, 0.46 mmol) were dissolved in 3 ml of \(N,N\)-dimethylformamide under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, the reaction mixture was stirred at 80 °C for 20 h. The solution was exposed to air, quenched with liquid nitrogen and the solvent was removed under reduced pressure.

**General Procedure for the Polymerization Using DTP-N\textsubscript{3}, as Exemplified for the Synthesis of PVBA\textsubscript{26}-N\textsubscript{3}**

DTP-N\textsubscript{3} (150 mg, 0.34 mmol), 4-styrenebenzoic acid (2.43 g, 16.4 mmol) and ACP (4.2 mg, 0.015 mmol) were dissolved in 5.5 ml of \(N,N\)-dimethylformamide under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, the reaction mixture was stirred at 80 °C for 4 h. The reaction mixture was exposed to air, quenched with liquid nitrogen and precipitated in toluene from THF/MeOH (50:50 v/v). After collecting the yellow precipitate by filtration, the product was dried under vacuum at 40 °C.
In order to analyze PVBA$_{26}$-N$_3$ via SEC, the benzenecarboxylic acid groups were methylated with MeOH, DCC, and DMAP.

**Yield:** 92 % (determined with $^1$H NMR)

$M_{n,NMR}$: 4500 g/mol

$M_{n,SEC}$: 2000 g/mol (in THF, calibration based on polystyrene standards)

$M_w/M_n$: 1.4 (determined with SEC)

$^1$H NMR (500 MHz, DMSO-$d_6$)

$\delta$ [ppm] = 0.6 - 0.8 (m, 3H, 1-H), 1.05 - 2.3 (m, 25H, 2-H to 12-H and 16-H to 17-H and 20-H to 22-H), 6.3 - 7.3 (m, 2H, 14-H), 7.3 - 8.0 (m, 2H, 13-H), 12.0 - 13.5 (m, 1H, 15-H).

$^1$H NMR (500 MHz, CDCl$_3$)

$\delta$ [ppm] = 0.6 - 0.8 (m, 3H, 1-H), 1.05 - 1.75 (m, 25H, 2-H to 12-H and 16-H to 17-H and 20-H to 22-H), 6.2 - 6.6 (m, 2H, 14-H), 8.1 - 8.5 (m, 2H, 13-H).

**General Procedure for the CuAAC with an Azide-Functionalized Polymer, as Exemplified for the Synthesis of PVBA$_{26}$-AAm**

PVBA$_{26}$-N$_3$ (200 mg, 0.044 mmol) and $N$-(prop-2-yn-1-yl)acrylamide (7 mg, 0.064 mmol) were dissolved in 1.5 ml of $N,N$-dimethylformamide. While the mixture was being stirred,
sodium ascorbate (0.88 mg, 0.0044 mmol) in 150 µl water was added, followed by CuSO₄·5 H₂O (1.1 mg, 0.0044 mmol) in 150 µl water. After the reaction mixture was stirred at r.t. for 3 d, the solvent was removed under reduced pressure, and the raw product was precipitated in n-hexane/ethyl acetate (60:40 v/v) from THF/MeOH (50:50 v/v). In order to remove copper, the polymer was dialyzed (MWCO = 1000 Da) in water for 3 d and then freeze-dried resulting in a yellow solid.

**Yield:** 175 mg (0.038 mmol, 86 %)

**Mₙ,NMR:** 4600 g/mol (determined with NMR)

**¹H NMR (500 MHz, DMSO-d₆)**
\[ \delta [\text{ppm}] = 0.6 - 0.8 (\text{m, 3H, 1-H}), 1.05 - 2.3 (\text{m, 25H, 2-H to 12-H and 16-H to 17-H 20-H to 21-H}), 4.15 (\text{m, 2H, 22-H}), 4.36 (\text{m, 2H, 24-H}), 5.58 (\text{m, 1H, 27-H}), 6.10 (\text{m, 1H, 27-H}), 6.24 (\text{m, 1H, 26-H}), 6.3 - 7.3 (\text{m, 2H, 14-H}), 7.3 - 8.0 (\text{m, 2H, 13-H}), 12.0 - 13.5 (\text{m, 1H, 15-H}). \]

**¹H NMR (500 MHz, DMSO-d₆)**
\[ \delta [\text{ppm}] = 0.6 - 0.8 (\text{m, 3H, 1-H}), 1.05 - 2.3 (\text{m, 25H, 2-H to 12-H and 16-H to 17-H 20-H to 22-H}), 4.19 (\text{m, 2H, 22-H}), 4.39 (\text{m, 2H, 24-H}), 5.59 (\text{m, 1H, 27-H}), 6.12 (\text{m, 1H, 27-H}), 6.26 (\text{m, 1H, 26-H}), 6.3 - 7.4 (\text{m, 2H, 14-H}), 7.8 - 8.9 (\text{m, 2H, 13-H}). \]
9.4 Conjugated Vinyl-Functionalization

Synthesis of 4-azidomethyl styrene

We adopted a method by O’Shea and colleagues.\cite{149} Vinylbenzyl chloride (1.5 g, 9.9 mmol) and sodium azide (1.3 g, 20 mmol) were dissolved in 5 ml of \( \text{N,N-dimethylformamide} \), and stirred at r.t. for 3 d. The reaction mixture was washed with brine and extracted with diethyl ether (3 \( \times \) 50 ml). The organic phase was dried over MgSO\(_4\) and the solvent was evaporated under reduced pressure resulting in a yellow oil.

Yield: 1.4 g (8.8 mmol, 89 %)

\( ^1\text{H NMR (500 MHz, DMSO-}d_6\text{)} \)

\( \delta \text{ [ppm]} = 4.43 \text{ (s, 2H, H-7)}, 5.28 \text{ (d, } J = 10.7 \text{ Hz, 1H, 1-H)}, 5.85 \text{ (d, } J = 17.7 \text{ Hz, 1H, 1-H)}, 6.75 \text{ (m, 1H, 2-H)}, 7.35 \text{ (d, } J = 8.2 \text{ Hz, 1H, 4-H)}, 7.5 \text{ (d, } J = 8.2 \text{ Hz, 2H, 5-H)}. \)

Synthesis of DTP-alkyne

DTP (1091 mg, 2.99 mmol) and PyBOP (1564 mg, 3.01 mmol) were dissolved in 3 ml of CH\(_2\text{Cl}_2\) under argon atmosphere. After being stirred at room temperature for 2 h, 2-propen-1-ylamine (166 mg, 3.01 mmol) and DIPEA (929 mg, 7.19 mmol) in 2 ml of CH\(_2\text{Cl}_2\) was added, and the reaction mixture was stirred for 3 d. The solvent was evaporated under reduced pressure and the raw product was purified by column chromatography using 0 - 24 % ethyl acetate in \( n\)-hexane (best separation effect at 16 %) resulting in yellow solid.

Yield: 1045 mg (2.6 mmol, 87 %)

\( \text{R}_f \text{ value: 0.24 (SiO}_2 \text{ / 20 % ethyl acetate)} \)
9.4 Conjugated Vinyl-Functionalization

$^1$H NMR (500 MHz, CDCl$_3$)

$\delta$ [ppm] = 0.89 (t, $J = 6.8$ Hz, 3H, 1-H), 1.21 - 1.35 (m, 16H, 2-H to 9-H), 1.35 - 1.43 (m, 2H, 10-H), 1.62 - 1.74 (m, 8H, 11H and 14H), 2.21 (t, $J = 3$ Hz, 20-H), 3.29 (t, $J = 7.4$ Hz, 12-H), (dd, $J = 5.2$ Hz, 2.7 Hz, 2H, 18-H), 6.6 (m, 1H, 17-H).

$^{13}$C NMR (126 MHz, CDCl$_3$)

$\delta$ [ppm] = 14.07 (CH$_3$, 1-C), 22.65 (CH$_2$, 2-C), 25.69 (CH$_3$, 14-C), 27.70 (CH$_2$, 11-C), 28.91 - 29.59 (7 × CH$_2$, 4-C to 10-C), 31.88 (CH$_2$, 3-C), 37.13 (CH$_2$, 12-C), 56.84 (C$_q$, 15-C), 71.66 (CH, 20-C), 79.22 (C$_q$, 19-C), 172.42 (C$_q$, 16-C), 219.65 (C$_q$, 13-C).

General Procedure for the Polymerization Using DTP-alkyne, as Exemplified for the Synthesis of PAA$_{53}$-alkyne

DTP-alkyne (150 mg, 0.38 mmol), AA (1.74 g, 24.15 mmol) and ACP (6.9 mg, 0.025 mmol) were dissolved in 8.2 ml of N,N-dimethylformamide under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, the reaction mixture was stirred at 70 °C for 4 h. The reaction mixture was exposed to air, quenched with liquid nitrogen and precipitated in toluene from THF/MeOH (50:50 v/v). After collecting the yellow precipitate by filtration, the product was dried under vacuum at 40 °C.

Yield: 84 % (determined with $^1$H NMR)

$M_{n,NMR}$: 4200 g/mol

$M_{n,SEC}$: 3700 g/mol (in 0.01 M NaH$_2$PO$_4$·H$_2$O + 0.5 M NaCl, calibration based on PAA standards)

$M_w/M_n$: 1.6 (determined with SEC)

$^1$H NMR (500 MHz, DMSO-$d_6$)

$\delta$ [ppm] = 0.86 (m, 3H, 1-H), 1.2 - 1.9 (m, 22H, 2-H to 12-H and 14-H), 2.0 - 2.3 (m, 1H, 13-H), 3.79 (m, 2H, 18-H).
General Procedure for the CuAAC with an Alkyne-Functionalized Polymer, as Exemplified for the Synthesis of PAA\textsubscript{53}-styrene

PAA\textsubscript{53}-alkyne (1 g, 0.24 mmol) and 4-azidomethyl styrene (100 mg, 0.63 mmol) were dissolved in 3 ml of N,N-dimethylformamide. While the mixture was being stirred, sodium ascorbate (26 mg, 0.13 mmol) in 600 µl water was added, followed by CuSO\textsubscript{4}•5 H\textsubscript{2}O (21 mg, 0.13 mmol) in 600 µl water. After the reaction mixture was stirred at r.t. for 3 d, the solvent was removed under reduced pressure, and the raw product was dialyzed (MWCO = 1000 Da) in water for 3 d and then freeze-dried resulting in a yellow solid.

Yield: 870 mg (0.20 mmol, 83 %)

\( M\text{\textsubscript{n,NMR}}: 4350 \text{ g/mol} \)

\( ^1H \text{ NMR} \ (500 \text{ MHz, D}_2\text{O}) \)
\( \delta \ [\text{ppm}] = 0.89 \ (m, \ 3H, \ H-1), \ 1.2 - 1.95 \ (m, \ 22H, \ 2-H \ to \ 12-H \ and \ 14-H), \ 2.0 - 2.45 \ (m, \ 1H, \ 13-H), \ 3.42 \ (m, \ 1H, \ 17-H), \ 4.46 \ (m, \ 2H, \ 18-H), \ 5.36 \ (m, \ 1H, \ 24-H), \ 5.62 \ (s, \ 2H, \ 20-H), \ 5.90 \ (m, \ 1H, \ 24-H), \ 6.82 \ (m, \ 1H, \ 23-H), \ 7.34 \ (m, \ 2H, \ 21-H), \ 7.55 \ (m, \ 2H, \ 22-H), \ 7.92 \ (s, \ 1H, \ 19-H). \)

9.5 Grafted Hydrogels Prepared in Organic Solvent

General Procedure Exemplified for the Synthesis of \textit{net-PNiPAAm-g-PVBA\textsubscript{26}-AAm Containing 1 mol-% PVBA\textsubscript{26}-AAm}

PVBA\textsubscript{26}-AAm (62 mg, 0.0125 mmol), NiPAAm (141.5 mg, 1.25 mmol), BIS (2.89 mg, 0.0188 mmol) and azobisisobutyronitrile (2.05 mg, 0.0125 mmol) were dissolved in 1 ml of pyridine under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, the reaction mixtures transferred into a glass tube of 3 mm diameter, sealed under argon and submerged in a water bath maintained at 70 °C. After the reaction was carried out for 1 d, the gel was separated from the glass tube and washed several times with water.
9.5 Grafted Hydrogels Prepared in Organic Solvent

Determination of the Swelling Degree

A hydrogel disk 5 mm in diameter was allowed to swell to equilibrium for 24 h in the solution of interest. The surface liquid was removed with a moistened filter paper and the sample was weighed. The disk was dried at 50 °C under reduced pressure, followed by recording the dry mass. The swelling degree $Q_m$ was described by:

$$Q_{m,i} = \frac{W_s}{W_d} - 1,$$

where $W_s$ is the weight of the swollen hydrogel and $W_d$ is the weight of the dried gel.

Determination of the Hydrogel Composition

Standard solutions composed of NiPAAm and VBA in 1 ml EtOH were prepared according to Table 9.1. All samples were measured through drop-casting from EtOH.

Table 9.1: Feed ratios of the prepared standard solutions.

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<td>VBA [mmol]</td>
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<tr>
<td>NiPAAm [mmol]</td>
<td>20.4</td>
<td>10.6</td>
<td>7.4</td>
<td>5.8</td>
<td>4.9</td>
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Determination of the Volume Phase Transition Temperature via Cloud-Point Method

A hydrogel disk 1 mm in diameter was allowed to swell to equilibrium for 24 h in distilled water and then placed between the inner side of a cuvette and a piece of quartz for vertical support. The cuvette was filled with distilled water. The temperature was ramped from 25 °C to 60 °C at a ramp rate of 5 °C by a hold for 15 minutes for 3 heating and cooling cycles. All measurements were carried out at 550 nm.

Determination of the Volume Phase Transition Temperature via Differential Scanning Calorimetry

A hydrogel disk 5 mm in diameter was allowed to swell to equilibrium for 24 h in distilled water. The temperature was ramped from 5 °C to 50 °C at a ramp rate of 1 °C for 2 heating cycles and 1 cooling cycle. VPTT was derived from the endothermic peak in the DSC plot (onset point).
9.6 Grafted Hydrogels Prepared in Water

General Procedure Exemplified for the Synthesis of $\text{net}-\text{PNiPAAm-g-PAA}_{53}$-styrene Containing 1 mol-% PAA$_{53}$-styrene

PAA$_{53}$-styrene (22.9 mg, 0.0062 mmol), NiPAAm (141.5 mg; 1.25 mmol) and BIS (2.89 mg, 0.0188 mmol) were dissolved in 0.8 ml of water (pH 10) under argon atmosphere. After the solution was degassed by several cycles of the freeze-pump-thaw procedure, potassium persulfate (2.05 mg, 0.0125 mmol) in 0.2 ml of water (pH 10) was added, followed by 1 µl of DIPEA. The reaction mixtures transferred into a glass tube of 3 mm diameter, sealed under argon and submerged in a water bath maintained at 10 °C. After the reaction was carried out for 1 d, the gel was separated from the glass tube and washed several times with water.

Determination of the Hydrogel Composition

Standard solutions composed of NiPAAm and AA in 1 ml EtOH were prepared according to Table 9.2. All samples were measured through drop-casting from EtOH.

<table>
<thead>
<tr>
<th>Table 9.2: Feed ratios of the prepared standard solutions.</th>
</tr>
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<tbody>
<tr>
<td>entry</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>AA [mmol]</td>
</tr>
<tr>
<td>NiPAAm [mmol]</td>
</tr>
</tbody>
</table>

Determination of the Corrected Cooperative Diffusion Coefficient

An air dried hydrogel sample was conditioned for 24 h in pH 9 buffer solution at 50 °C. The sample was transferred into a petri dish with pH 9 buffer solution at r.t. in which the hydrogel swelled until equilibrium was reached. In order to follow the swelling process, optical microscope images were recorded every 10 minutes. The characteristic time $\tau$ was obtained by $\tau = \frac{1}{k}$ from the slope $k$ of the curve for $t >> 0$ in the plot of the logarithm of $\Delta d(t)/\Delta d(t_0)$ versus time.$^{[125]}$
Rheological Analysis

All samples were allowed to swell to equilibrium for 24 h in pH 9 buffer solution. Before conducting the rheological analysis, the surface liquid was removed with a moistened filter paper.

Photopolymerization, General Procedure Exemplified for the Synthesis of net-PNiPAAm-g-PAA_{53}-styrene Containing 1 mol-% PAA_{53}-styrene

PAA_{53}-styrene (22.9 mg, 0.0062 mmol), NiPAAm (141.5 mg; 1.25 mmol), LAP (3.68 mg, 0.0125 mmol) and BIS (2.89 mg, 0.0188 mmol) were dissolved in 1 ml of water (pH 10). The reaction mixtures transferred into a glass tube and cooled in an ice-bath during irradiation. The light intensity of the UV lamp was set to 0.3 W/cm\(^3\) (intensity 1 %).

9.7 Tetra-Responsive Chemo-Mechanical Valve

Actuator Chamber

Dried and crushed hydrogel particles were sandwiched between two filter disks (diameter: 10 mm) inside a rubber hose (inside diameter: 9 mm, wall thickness: 2 mm). The hydrogel layer was 1 mm or 4 mm, which was determined with microscope images.

Hydrogel Particles

Net-PNiPAAm-g-PAA_{53}-styrene containing 0.6 mol-% PAA_{53}-styrene was synthesized as described in chapter 9.6 and crushed with a mortar and pestle.

Fluidic Test Station

A cylindrical separatory funnel was used as a solvent reservoir. The actuator chamber was connected with the outflow of the solvent reservoir.

9.8 Chemo-Fluidic Membrane Transistor

The CFMT was fabricated with P. Frank according to literature.\(^{[44]}\)
**Master Mold**

A glass substrate with a thickness of 700 microns was cleaned with acetone, isopropanol as well as distilled water and dried using nitrogen. After the glass substrate was baked at 150 °C for 20 min on a hot plate, one layer resist was laminated and soft baked at 85 °C for 3 min. The lamination process was repeated for a second layer. The substrate was irradiated with UV light (intensity: 1.66 W/cm²) through a photomask for 90 s for the flow layer and 65 s for the control layer. The substrates were subjected to a post exposure bake at 85 °C for 30 min. The resist was developed with the Ordyl developer and rinser. The substrate was washed with isopropanol and distilled water and hard baked at 120 °C for 1.5 h.

**Flow and Control Layer**

**Flow Layer**

PDMS (27.5 g) in a ratio 10:1 was mixed with an electric stirrer for 5 min and degassed for 45 min. The bubble-free PDMS was poured onto the flow master to obtain the flow layer with a thickness of 4 mm. The flow layer was cured in a 60 °C convection oven for 2 h.

**Control Layer**

PDMS (22.0 g) in a ratio 10:1 was mixed with an electric stirrer for 5 min and degassed for 45 min. The bubble-free PDMS was spin-coated onto the control master to obtain the control layer with a thickness of 130 microns. The control layer was cured in a 60 °C convection oven for 2 h.

**Hydrogel Particle**

PAA\textsubscript{53}-styrene (211.5 mg, 0.0503 mmol), NiPAAm (707.3 mg; 6.25 mmol), 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (14 mg, 0.064 mmol) and BIS (14.3 mg, 0.093 mmol) were dissolved in 5 ml of water (pH 10). After the solution was degassed by purging with argon for 30 min, the reaction mixture was transferred under argon atmosphere in a reaction chamber and closed with a glass substrate. The closed reaction chamber was sandwiched between a PET plate and a photomask. The reaction chamber was irradiated with UV light through the photomask for 2 min. The hydrogel particles were dried at 60 °C and stored in a 50 vol-% water/isopropanol solution.
Final Device

The flow layer was peeled off the master. Flow and control layer (still on the master) were exposed to an oxygen plasma at 50 W for 2 min with an oxygen flow of 20 sccm. After the flow layer was inhibited from bonding at the channel barrier by dispensing a small drop of oil on top, both layers were aligned accordingly. The fluidic connections were punched with a biopsy tool (diameter: 1.2mm). After the hydrogel particle was incorporated between the posts in the control layer, the chip was bonded onto a glass substrate via oxygen plasma activation (50 W for 2 min with an oxygen flow of 20 sccm).

Measuring Set-up

The flow channel inlet was supplied by a pressure flow pump utilizing a pressure controller controlled by a fieldbus system,[186] while the outlet was set to ambient pressure. Distilled water was used as the fluid. The flow rate was measured with a flow sensor providing a flow range from 0 to 1000 µl/min. The control channel was supplied with a syringe pump at a constant flow of 10 µl/min with the solution of interest.
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5.1 Synthesis of net-PNiPAAm, net-PNiPAAm-g-PAA29-styrene, net-PNiPAAm-g-PAA53-styrene and net-PNiPAAm-g-PAA75-styrene.

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<tbody>
<tr>
<td>2D-DOSY NMR</td>
<td>Diffusion-ordered spectroscopy nuclear magnetic resonance</td>
</tr>
<tr>
<td>4VP</td>
<td>4-Vinylpyridine</td>
</tr>
<tr>
<td>AA</td>
<td>Acrylic acid</td>
</tr>
<tr>
<td>AAPBA</td>
<td>3-(Acrylamido)-phenylboronic acid</td>
</tr>
<tr>
<td>ACP</td>
<td>4,4′-Azobis(4-cyanopetanoyl)</td>
</tr>
<tr>
<td>AIBN</td>
<td>Azobisisobutyronitrile</td>
</tr>
<tr>
<td>AM</td>
<td>Acrylamide</td>
</tr>
<tr>
<td>AMA</td>
<td>Allyl methacrylate</td>
</tr>
<tr>
<td>AN</td>
<td>Acrylonitrile</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulfate</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated total reflection</td>
</tr>
<tr>
<td>ATRP</td>
<td>Atom transfer radical polymerization</td>
</tr>
<tr>
<td>BIS</td>
<td>N,N′-Methylenebisacrylamide</td>
</tr>
<tr>
<td>CFMT</td>
<td>Chemo-fluidic membrane transistor</td>
</tr>
<tr>
<td>CRP</td>
<td>Controlled radical polymerization</td>
</tr>
<tr>
<td>CTA</td>
<td>Chain transfer agent</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper-catalyzed alkyne-azide cycloaddition</td>
</tr>
<tr>
<td>DAMA</td>
<td>N-(N′,N′-Dicarboxymethylaminopropyl)methacrylamide</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>------------------------------------------------</td>
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<td>DEAAm</td>
<td>$N,N$-Diethylacrylamide</td>
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<tr>
<td>DEAEMA</td>
<td>$N,N$-Diethylaminoethyl methacrylate</td>
</tr>
<tr>
<td>DIPEA</td>
<td>$N,N'$-Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAEMA</td>
<td>$N$-2-(dimethylamino)ethyl methacrylamide</td>
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<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DOPA</td>
<td>3,4-Dihydroxyphenylalanine</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>DTP</td>
<td>2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid</td>
</tr>
<tr>
<td>DTP-alkyne</td>
<td>Dodecyl (2-methyl-1-oxo-1-(prop-2-yn-1-ylamino)propan-2-yl) carbonothioylthioate</td>
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<tr>
<td>DTP-allyl amide</td>
<td>1-(Allylamino)-2-methyl-1-oxopropan-2-yl dodecyl carbonothioylthioate</td>
</tr>
<tr>
<td>DTP-allyl ester</td>
<td>Allyl 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid</td>
</tr>
<tr>
<td>DTP-N$_3$</td>
<td>1-((3-Azidopropyl)amino)-2-methyl-1-oxopropan-2-yl dodecyl carbonothioylthioate</td>
</tr>
<tr>
<td>DTP-vinyl ester</td>
<td>But-3-en-1-yl 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid</td>
</tr>
<tr>
<td>EGF</td>
<td>End-group functionalization</td>
</tr>
<tr>
<td>FRP</td>
<td>Free radical polymerization</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GTA</td>
<td>Glutaraldehyde</td>
</tr>
<tr>
<td>HEMA</td>
<td>2-Hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>His</td>
<td>Histidine</td>
</tr>
<tr>
<td>HPMAM</td>
<td>$N$-2-Hydroxypropyl methacrylamide</td>
</tr>
<tr>
<td>HRP</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>IPN</td>
<td>Interpenetrating network</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
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<td>LAP</td>
<td>Lithium phenyl-2,4,6-trimethylbenzoylphosphinate</td>
</tr>
<tr>
<td>MA</td>
<td>Methyl acrylate</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy</td>
</tr>
<tr>
<td>MMA</td>
<td>Methyl methacrylate</td>
</tr>
<tr>
<td>NiPAAm</td>
<td>N-Isopropylacrylamide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>NVC</td>
<td>N-Vinylcarbazole</td>
</tr>
<tr>
<td>NVP</td>
<td>N-Vinylpyrrolidone</td>
</tr>
<tr>
<td>P4VP-AAm</td>
<td>Acrylamide-functionalized poly(4-vinylpyridine)</td>
</tr>
<tr>
<td>P4VP-N₃</td>
<td>Azide-functionalized poly(4-vinylpyridine)</td>
</tr>
<tr>
<td>PAA</td>
<td>Poly(acrylic acid)</td>
</tr>
<tr>
<td>PAA-alkyne</td>
<td>Alkyne-functionalized poly(acrylic acid)</td>
</tr>
<tr>
<td>PAA-allyl amide</td>
<td>Allyl amide-functionalized poly(acrylic acid)</td>
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<td>Allyl ester-functionalized poly(acrylic acid)</td>
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<tr>
<td>PAA-N₃</td>
<td>Azide-functionalized poly(acrylic acid)</td>
</tr>
<tr>
<td>PAA-styrene</td>
<td>Styrene-functionalized poly(acrylic acid)</td>
</tr>
<tr>
<td>PAA-vinyl ester</td>
<td>Vinyl ester-functionalized poly(acrylic acid)</td>
</tr>
<tr>
<td>PDMAEMA</td>
<td>Poly-((2-dimethylamino)ethyl methacrylamide)</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
</tr>
<tr>
<td>PMAA</td>
<td>Poly(methacrylic acid)</td>
</tr>
<tr>
<td>PMEDTA</td>
<td>N,N,N′,N″,N‴-Pentamethyldiethylenetriamine</td>
</tr>
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>PVBA-AAm</td>
<td>Acryl amide-functionalized poly(4-vinylbenzoic acid)</td>
</tr>
<tr>
<td>PVBA-N₃</td>
<td>Azide-functionalized poly(4-vinylbenzoic acid)</td>
</tr>
<tr>
<td>PyBOP</td>
<td>Benzotriazol-1-ylxy-tripyrrolidino-phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>r.t.</td>
<td>Room temperature (22 °C)</td>
</tr>
<tr>
<td>Rabbit IgG</td>
<td>Rabbit immunoglobulin G</td>
</tr>
<tr>
<td>RAFT</td>
<td>Reversible addition-fragmentation chain-transfer</td>
</tr>
<tr>
<td>Semi-IPN</td>
<td>Semi-interpenetrating polymer network</td>
</tr>
<tr>
<td>SPAA</td>
<td>Spirobenzopyran-functionalized acrylic acid</td>
</tr>
<tr>
<td>SPAAC</td>
<td>Strain-promoted alkyne-azide cycloaddition</td>
</tr>
<tr>
<td>SPS</td>
<td>Sodium persulfates</td>
</tr>
<tr>
<td>SRMP</td>
<td>Stable-radical-mediated polymerization</td>
</tr>
<tr>
<td>St</td>
<td>Styrene</td>
</tr>
<tr>
<td>TGase</td>
<td>Transglutaminase</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TMEDA</td>
<td>N,N,N',N'-Tetramethylethane-1,2-diamine</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>UV/Vis</td>
<td>Ultraviolet–visible spectroscopy</td>
</tr>
<tr>
<td>VAc</td>
<td>Vinyl acetate</td>
</tr>
<tr>
<td>VBA</td>
<td>4-Vinylbenzoic acid</td>
</tr>
<tr>
<td>VCl</td>
<td>Vinylcaprolactam</td>
</tr>
<tr>
<td>VI</td>
<td>Vinyl imidazole</td>
</tr>
<tr>
<td>VME</td>
<td>Vinylimethylether</td>
</tr>
<tr>
<td>VPTT</td>
<td>Volume phase transition temperature</td>
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</table>
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Conference Contributions


Appendix

**Figure 9.1:** MALDI-TOF spectra of PVBA$_{26}$-N$_3$ and P4VP$_{38}$-N$_3$.

**Figure 9.2:** FTIR spectra of the standard solutions and calibration curve to determine the hydrogel composition of net-PNiPAAm-g-PVBA$_{26}$-AAm.
Figure 9.3: (left) Equilibrium swelling degree $Q_{m,T}$ for $\text{net-PNiPAAm-g-P4VP}_{38}$-$\text{AAm}$ samples with different grafting densities in pH 9 (bottom) and pH 3 buffer solution (top) plotted as a function of temperature. (right) Determined temperature response ratio $Q_{m,T}$ for $\text{net-PNiPAAm-g-P4VP}_{38}$-$\text{AAm}$ samples with different grafting densities. The temperature sensitivity retains with a suitable response for all samples (color code in the right part of the figure corresponds to the composition of the curves in the left part).
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Figure 9.5: ATR-IR spectra of net-PNiPAAm-g-PAA₅₃-styrene samples with different grafting densities.

Figure 9.6: FTIR spectra of the standard solutions and calibration curve to determine the hydrogel composition of net-PNiPAAm-g-PAA₅₃-styrene.
Table 9.3: Data of the swelling kinetics for determining the corrected cooperative diffusion coefficient $D_{\text{coop}}$.

<table>
<thead>
<tr>
<th>PAA$_{53}$-styrene content [mol-%]</th>
<th>sample</th>
<th>$l$ [mm]</th>
<th>$\tau$ [s]</th>
<th>$h$</th>
<th>$D_{\text{coop}}$ [cm/s]</th>
<th>corr. $D_{\text{coop}}$ [cm/s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0.728</td>
<td>161045.8</td>
<td>0.752</td>
<td>3.334 \cdot 10^{-7}</td>
<td>4.434 \cdot 10^{-7}</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0.725</td>
<td>172364.3</td>
<td>0.836</td>
<td>3.094 \cdot 10^{-7}</td>
<td>3.625 \cdot 10^{-7}</td>
</tr>
<tr>
<td>0.25</td>
<td>3</td>
<td>0.805</td>
<td>154236.9</td>
<td>0.853</td>
<td>4.257 \cdot 10^{-7}</td>
<td>4.991 \cdot 10^{-7}</td>
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<tr>
<td>0.25</td>
<td>1</td>
<td>0.728</td>
<td>31579.0</td>
<td>0.925</td>
<td>1.701 \cdot 10^{-6}</td>
<td>1.838 \cdot 10^{-6}</td>
</tr>
<tr>
<td>0.25</td>
<td>2</td>
<td>0.761</td>
<td>37636.4</td>
<td>0.914</td>
<td>1.560 \cdot 10^{-6}</td>
<td>1.707 \cdot 10^{-6}</td>
</tr>
<tr>
<td>0.25</td>
<td>3</td>
<td>0.664</td>
<td>42553.2</td>
<td>0.942</td>
<td>1.051 \cdot 10^{-6}</td>
<td>1.091 \cdot 10^{-6}</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>0.525</td>
<td>16620.5</td>
<td>0.963</td>
<td>1.679 \cdot 10^{-6}</td>
<td>1.743 \cdot 10^{-6}</td>
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<tr>
<td>0.5</td>
<td>2</td>
<td>0.529</td>
<td>11592.0</td>
<td>0.972</td>
<td>2.441 \cdot 10^{-6}</td>
<td>2.511 \cdot 10^{-6}</td>
</tr>
<tr>
<td>0.5</td>
<td>3</td>
<td>0.529</td>
<td>10118.0</td>
<td>0.975</td>
<td>2.697 \cdot 10^{-6}</td>
<td>2.766 \cdot 10^{-6}</td>
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<td>1</td>
<td>1</td>
<td>0.673</td>
<td>12494.0</td>
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<td>3.668 \cdot 10^{-6}</td>
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<td>1</td>
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<td>0.593</td>
<td>10443.9</td>
<td>0.910</td>
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<td>3.754 \cdot 10^{-6}</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.612</td>
<td>10787.2</td>
<td>0.896</td>
<td>3.518 \cdot 10^{-6}</td>
<td>3.925 \cdot 10^{-6}</td>
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Figure 9.7: Storage modulus for net-PNiPAAm-$g$-PAA$_{53}$-styrene containing 1 mol-% PAA-styrene samples plotted as a function of the angular velocities. The storage modulus in chapter 5.6 discussed was calculated by the average storage modulus of three samples at $\omega = 1 \text{ rad/s}$.\cite{43}
**Figure 9.8:** Optical microscope images of net-PNiPAam-g-PAA<sub>53</sub>-styrene samples with different grafting densities in the swollen state (r.t. and pH 9) and in the collapsed state after applying the pH (r.t. and pH 3) or temperature stimulus (50 °C and pH 9).<sup>[43]</sup>
Figure 9.9: Uncorrected and corrected flow rate plotted as a function of the outflow. The solution alternates between pH 9 buffer solution at r.t. and one solution in which the hydrogel collapses (e.g. pH 3 buffer solution, pH 9 buffer solution at 50 °C, 40 vol.% EtOH solution, or 1 mol/L NaCl solution). The color background of the diagram represents the provided solution. An actuator chamber with a size of 10 × 1 mm filled with crushed particles of net-PNiPAAm-g-PAA₃₃-styrene containing 0.6 mol-% PAA₃₃-styrene was used in this experiment.
Figure 9.10: Microscope image of the swollen net-PNiPAAm-g-PAA₅₃-styrene hydrogel containing 0.6 mol-% PAA₅₃-styrene in the CFMT.
Acknowledgements

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Versicherung

Hiermit versichere ich, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe; die aus fremden Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

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Frühere Promotionsverfahren haben nicht stattgefunden.

Ich erkenne die Promotionsordnung der Fakultät Mathematik und Naturwissenschaften der Technischen Universität Dresden vom 23.02.2011 inklusive der Änderungen vom 15.06.2011 und 18.06.2014 in vollem Umfang an.

David Gräfe
Dresden, den