Freie Universität Berlin

Functional Polymeric Toolkits: From Supramolecular to Hybrid Polymer Gels

DISSERTATION

zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat.)

Fachbereich Biologie, Chemie, Pharmazie

vorgelegt von

Sebastian Hackelbusch, M.Sc.

aus Berlin

Dezember 2015

Diese Arbeit wurde unter Leitung von Prof. Dr. Sebastian Seiffert im Zeitraum von Oktober 2011 bis Dezember 2015 am Institut für Chemie und Biochemie der Freien Universität angefertigt.

1. Gutachter: Prof. Dr. Sebastian Seiffert

2. Gutachter: Prof. Dr. Rainer Haag

Disputation am: 09.03.2016

Acknowledgements

First, I would like to thank Prof. Dr. Sebastian Seiffert for giving me the opportunity to conduct research on this interesting topic and for his scientific support throughout the last years. Furthermore, Prof. Dr. Rainer Haag is gratefully acknowledged for co-refereeing this thesis and for financial support.

Moreover, I would like to thank all the members of the Seiffert-, Haag-, Ballauf-, Tzschucke-, Oppermann-, and Caldéron-group for interesting scientific discussions. Special thanks go to Prof. Dr. Wilhelm Oppermann for allowing me to use the confocal laser scanning microscope for the fluorescence recovery after photobleaching studies in Clausthal. Additionally, I would like to thank Dr. Christian Secker for numerous scientific discussions and help with SEC measurements.

I would like to acknowledge my cooperation partners Prof. Dr. David Weitz, Dr. Dirk Steinhilber, Dr. Nora Traulsen and Dr. Stefanie Wedepohl.

The following undergraduate and graduate students that were working under my supervision are kindly thanked: Jonas Schröder, Peter van Assenbergh, Hendrik Liebe, and Maria Baumann.

Furthermore, I appreciate the NMR and MS facilities of the Institute of Chemistry and Biochemistry for their numerous measurements and Marlies Graewert (MPI, Golm) for performing SEC analyses. A special thanks goes to the staff of the material store. I would also like to thank Jutta Hass, Lisa Hummel, and Brigitte Behrmann-Harris for taking care of all administrative issues.

My friend and colleague Dr. Torsten Rossow is thanked for the excellent atmosphere in the lab, his support and help as well as proofreading of the manuscript.

Financial support from the Focus Area NanoScale and the Fund of the Chemical Industry (Germany) is gratefully acknowledged.

Finally, I would like to thank my parents and my grandmother for their support throughout my whole education.

Contents

List of Abbreviations and Symbols

BIP	2,6-bis(1-methylbenzimidazolyl)pyridine
BZ	Belousov–Zhabotinsky
DAN	2,7-diamido-1,8-naphthyridine
DLS	dynamic light scattering
DOPA	dihydroxy-phenylalanine
EDTA	ethylenediaminetetraacetic acid
GG	gellan gum
HEEDTA	hydroxyethyl ethylenediaminetriacetic acid
HEMA	hydroxyethyl methacrylate
HPMA	*N*-(2-hydroxypropyl)-methylacrylamide
ITC	isothermal titration calorimetry
MASI	*N*-(methacryloyloxy)succinimide
NIPAM	*N*-isopropylacrylamide
NMR	nuclear magnetic resonance
PAAm	poly(acrylamide)
PAMPS	poly(2-acrylamido-2-methylpropanesulfonic acid)
P(AN-*co*-MPC)	poly(acrylonitrile-*co*-2-methacryloyloxyethyl phosphorylcholine)
PEG	poly(ethylene glycol)
PHPMA	poly(*N*-(2-hydroxypropyl)-methylacrylamide)
PLGA	poly(lactide-*co*-glycolide)
PNIPAM	poly(*N*-isopropylacrylamide)
PPE	poly(*p*-phenylene ethynylene)
PS	polystyrene
PVC	poly(vinyl chloride)
SEC	size exclusion chromatography
SEM	scanning electron microscopy
THF	tetrahydrofuran

UG	ureidoguanosine
Upy	2-ureido-4-pyrimidinone
UV	ultraviolet

1. Introduction

Parts of this introduction have already been published previously in the context of reference [1] by the author and his supervisor. Polymeric gels are three-dimensional networks that are swollen in organic solvents or water. Commonly, these gels are categorized into chemical and physical gels, depending on the type of crosslinking used to achieve chain interconnection.[2] Chemical networks are crosslinked by covalent bonds that are irreversible on experimental timescales. Such networks can be realized by the reaction of functionalized precursor polymers with each other by, e.g. Schiff-base formation,[3–5] Michael-type addition,[4,6] and 'click' chemistry,[7] or by (co)polymerization of suitable multifunctional monomers.[3,8–10] The mechanical properties of such gels are determined by their crosslinking density and chain flexibility.[11] Depending on the desired application, the resulting material characteristics can range from soft and weak for biomedical or optical purposes[8,10] to hard and tough for engineering and medical applications.[12–15] However, the high crosslink density in hard gels often entails a pronounced susceptibility to bond breakage by extensive mechanical stress as well as opacity, thereby impairing the utility of such materials.[16–17]

A feasible alternative to chemical gels are physical gels, wherein which the interconnection of chains is achieved by reversible supramolecular assembly of complementary functional groups that are attached to the polymer chains; as a result, these crosslinks can break and rearrange on experimental timescales.[18] To form such physical crosslinks, hydrogen bonding,[19–20] metal complexation,[21–22] ionic,[23–24] or hydrophobic interactions[25–27] can be used. As a consequence of the varying strength of these non-covalent interactions, as summarized in Figure 1,[28–29] the mechanical properties of supramolecular polymer networks can be adjusted to their desired applications.

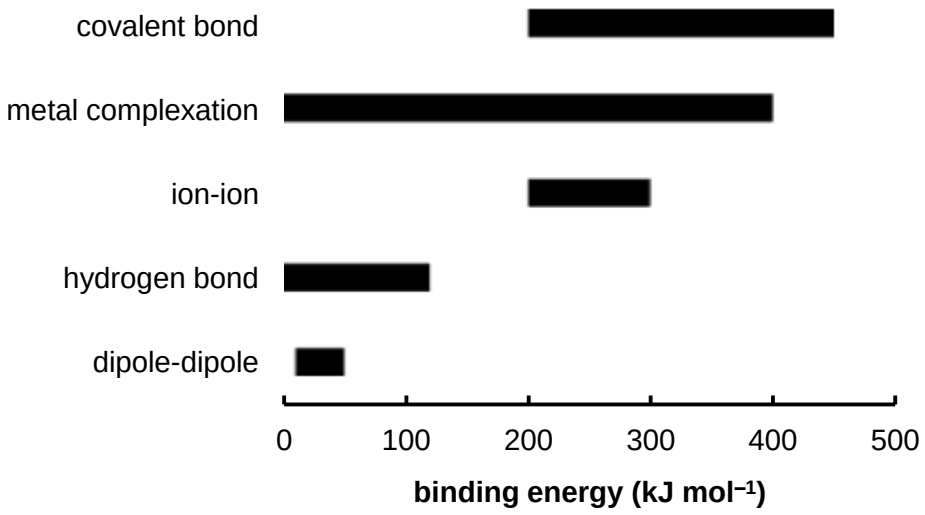


Figure 1. Overview of the binding strength of the non-covalent interactions in relation to covalent bonding discussed in this thesis. Modified with permission from ref. 28. Copyright 2015 Springer International Publishing AG.

Physically crosslinked polymer gels exhibit two unique abilities: self-healing[30–31] and shear-thinning.[18] Self-healing describes the effect that after gel rupturing, the free functional moieties

tend to reassociate with each other upon contact, thereby allowing the gels to regain their initial mechanical properties. This effect is applied in self-healing coatings,[32–34] concretes,[35] and in the formation of composite materials.[36–38] At shear-thinning, the viscosity of physical gels is decreased upon application of mechanical stress, due to the disruption of the supramolecular bonds. This gives rise to the implantation of supramolecular gels via syringe, whereupon their mechanical properties are restored. Additionally, shear-thinning allows for the molding of supramolecular materials into different shapes in engineering applications.[39] Moreover, supramolecular crosslinking features another characteristic: physical gels can be degraded into their polymer building blocks by external stimulation such as change of pH,[40–41] temperature,[42-43] solvent composition,[44] and/or addition of chelating agents.[35,45] This stimuli-responsiveness makes these materials suitable candidates for applications such as in drug delivery,[46–48] wound dressing,[49–50] and tissue engineering.[51–54]

The combination of both physical interactions and chemical crosslinking gives rise to new materials that exhibit unique abilities such as shape-memory,[55–57] stimuli-switchability,[58–60] and self-healing after extensive strain,[61–62] while exhibiting tough mechanical properties. These hybrid materials are thereby broadening the potential applications of polymeric gels.

For the preparation of gels, different precursor polymers can be used, including biopolymers, synthetic polymers, and composites of both. Many naturally occurring polymers such as alginate,[49,52,63] gelatin,[64–67] or chitosan[46,52,68–69] are able to form hydrogels without the necessity of any preceding functionalization. Moreover, these precursors are biocompatible, biodegradable, bioavailable, and most importantly cheap, which makes them interesting candidates for biomedical applications. Because these materials are derived from living organisms, however, every batch of polymer is slightly different from another and the manufacturing of large amounts of polymers is therefore challenging.[70–73] As an alternative, synthetic polymers, such as poly(ethylene glycol),[52,74–75] poly(*N*-(2-hydroxypropyl) methacrylamide),[59–60,76] and polyglycerol[77–79] can be used. These polymers need to be chemically functionalized to form gels entailing an increase of the costs and the workload. However, this allows also for rational material design along with the ability to create tailor-made materials for more specific applications. A further alternative for material design is based on a composite of both natural and synthetic polymers, combining the utility of both.[80–81]

The following introduction will cover the current scientific state of the art of supramolecular polymer gels with a focus on hydrogen bonding, metal complexation, and ionic interactions. Furthermore, the potential of hybrid hydrogels for applications, such as drug delivery, self-healing high performance materials, and actuators will be highlighted.

1.1. Supramolecular Polymer Gels

When Lehn, Cram, and Pederson were awarded the chemistry Nobel prize *"for their development and use of molecules with structure-specific interactions of high selectivity"* in 1987, the interest in supramolecular chemistry was growing.[82] Nowadays, almost 30 years later, supramolecular chemistry has developed into a broad and active field of research including newly formed subgroups that deal with more specific topics such as supramolecular polymers,[22,83–86] self-assembled architectures,[87–88] and supramolecular polymer gels.[89–90] The main differences in the research of these subgroups are the utilized precursor molecules and the length scale of the formed supramolecular assemblies. Low molecular bifunctional precursors typically form supramolecular polymer chains on a nanometer sized length scale, whereas functionalized macromolecular precursors form supramolecular polymer gels that can assemble into aggregates in the range of up to

micrometer size. These assemblies also have an impact on the characteristics and utility of these materials; supramolecular polymers tend to be brittle and hard to customize. In contrast, supramolecular polymer gels can range from very soft to extremely resilient. Additionally, the use of functionalized precursor polymers allows for the customization of the resulting gels by controlling the type of supramolecular crosslinking (e.g. hydrogen bonding, metal complexation, etc.) and the degree of functionalization.

There are two possible approaches to form physical crosslinks in supramolecular polymer gels: a homo- and hetero-complementary approach.[28] In the homo-complementary approach motifs are used that can associate directly with each other. Even though this principle is straight forward, the material properties are difficult to tune and the sample preparation is often quite challenging and requires harsh reaction conditions. These drawbacks can be overcome by hetero-complementary systems, wherein which two different motifs are used that can form supramolecular assemblies with each other. By this means, the binding strength and the resulting material properties can be precisely adjusted. Because the functional moieties are attached to polymer chains, different design principles are conceivable to form gels, as compiled in Figure 2. In two principles, the polymer chain ends are functionalized and a network is formed by association of these chain ends. If a system is used that has a functionality higher than two, the network is directly formed by the association of these motifs, as shown in Figure 2A. By contrast, systems that have a functionality of two can form only linear supramolecular polymer chains and loops. In this case, gelation occurs by chain entanglement or lateral interactions of the already formed assemblies with each other, as a result of clustering, crystallization, and/or stacking, as visualized in Figure 2B. In a third principle, the supramolecular motifs are attached along the polymer chain. The polymer gels are formed independent of the functionality of the motifs, as far as more than two functional groups are present per polymer chain. This principle can be applied either by direct association of polymers bearing hetero-complementary supramolecular moieties (Figure 2C) or by using low-molecular weight crosslinkers (Figure 2D). The latter example has the advantage that only one kind of motif needs to be present on the polymer chains and gelation only occurs after the crosslinker is added, allowing for a much more precise preparation of samples. Low-molecular weight crosslinkers can also be used for the end-functionalized polymer chains in the first two design principles.

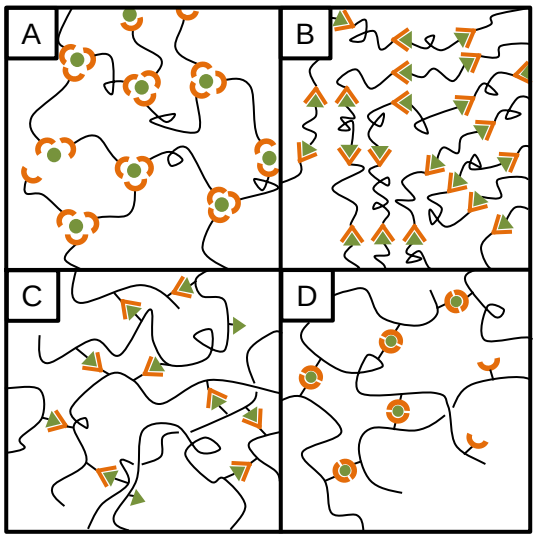


Figure 2. Overview of different design principles to form supramolecular polymer networks based on hetero-complementary assembly. Endgroup-functionalized linear polymer chains can form crosslinks by (A) employing motifs with a functionality higher than two or by (B) using motifs with a functionality of two and additional lateral chain interactions. Moreover, side-chain functionalization can be utilized for the formation of supramolecular crosslinking by (C) mutual hetero-complementary polymer–polymer binding or (D) addition of suitable low molecular crosslinkers. Adopted from ref. 28. Copyright 2015 Springer International Publishing AG.

For the preparation of supramolecular networks by these design principles, the supramolecular crosslinkable groups needs to be introduced into the precursor polymers. This can be achieved either by post-polymerization functionalization of macromolecular precursors or by using functional supramolecular crosslinkable comonomers in the polymerization step. The second approach gives rise to *in situ* gelling supramolecular polymers or to functional polymer precursors that can be used directly after preparation to form polymer gels.

In the following, examples of recent work on supramolecular polymer gels crosslinked by hydrogen bonding, metal complexation, and ionic interactions will be discussed.

1.1.1. Hydrogen Bonding

Hydrogen bonding is the most prominent supramolecular interaction in Nature, playing a crucial role in many biological processes including DNA/RNA base pairing, enzyme catalysis, protein folding, and molecular recognition. In recent studies of synthetic supramolecular networks, this non-covalent interaction has also been widely used.[91–94] Even though the binding strength of hydrogen bonds is rather low with 4–120 kJ mol^{-1} compared to metal complexation and ionic interactions, their directionality, versatility, and most importantly their multiplicity negate this drawback easily.

Hydrogen bonds are the non-covalent connection of two atoms mediated by a hydrogen atom; both atoms need to be more electronegative than hydrogen. One of these atoms has to be bound to a hydrogen atom and is thereby referred to as the proton donor (D). The other highly electronegative atom is called the proton acceptor (A).[95] The strength of a single hydrogen bond is depending on the dipole moment of the donor–acceptor system as well as the solvent. Moreover, the association strength of a motif of multiple hydrogen bonds is depending on the fashion these hydrogen bonds are acting together. If this occurs in a cooperative way, the binding strength increases much more than their simple numerical sum. Jorgensen and coworkers investigated this

effect for three different hydrogen bonding complexes, as shown in Figure 3.[96–98] Complexes of an ADA–DAD type exhibit association constants, K_a, of 10^2 M^{-1} in chloroform, whereas DAA–ADD complexes possess constants of 10^4 M^{-1}. The highest association constant in these trials showed an AAA–DDD array with values exceeding 10^5 M^{-1}. Subsequent extensive calculations showed that this effect can be attributed to secondary interactions of the donor–acceptor system. Diagonally opposed sites repel each other when they are of the same kind, whereas dissimilar sites associate with each other. Accordingly, the AAA–DDD array with four attractive secondary interactions exhibits the largest association constant and the DAA–ADD array with four repulsive interactions exhibits the lowest association constant.

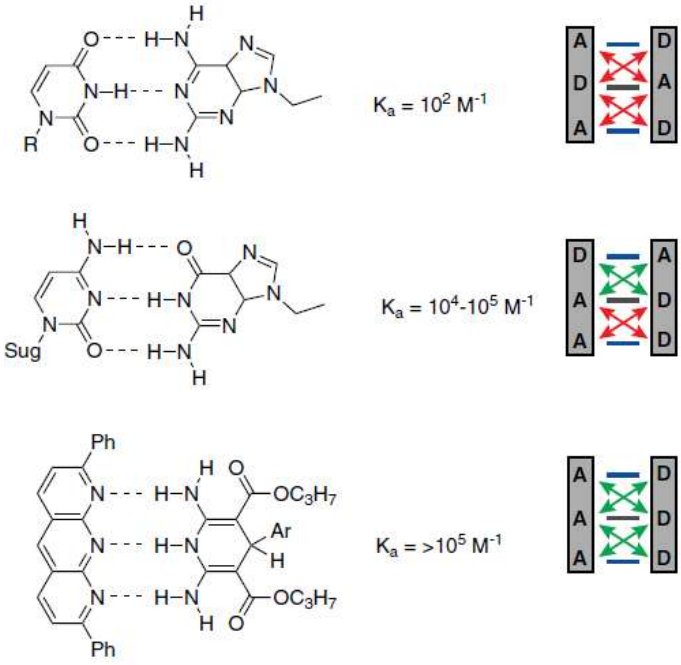
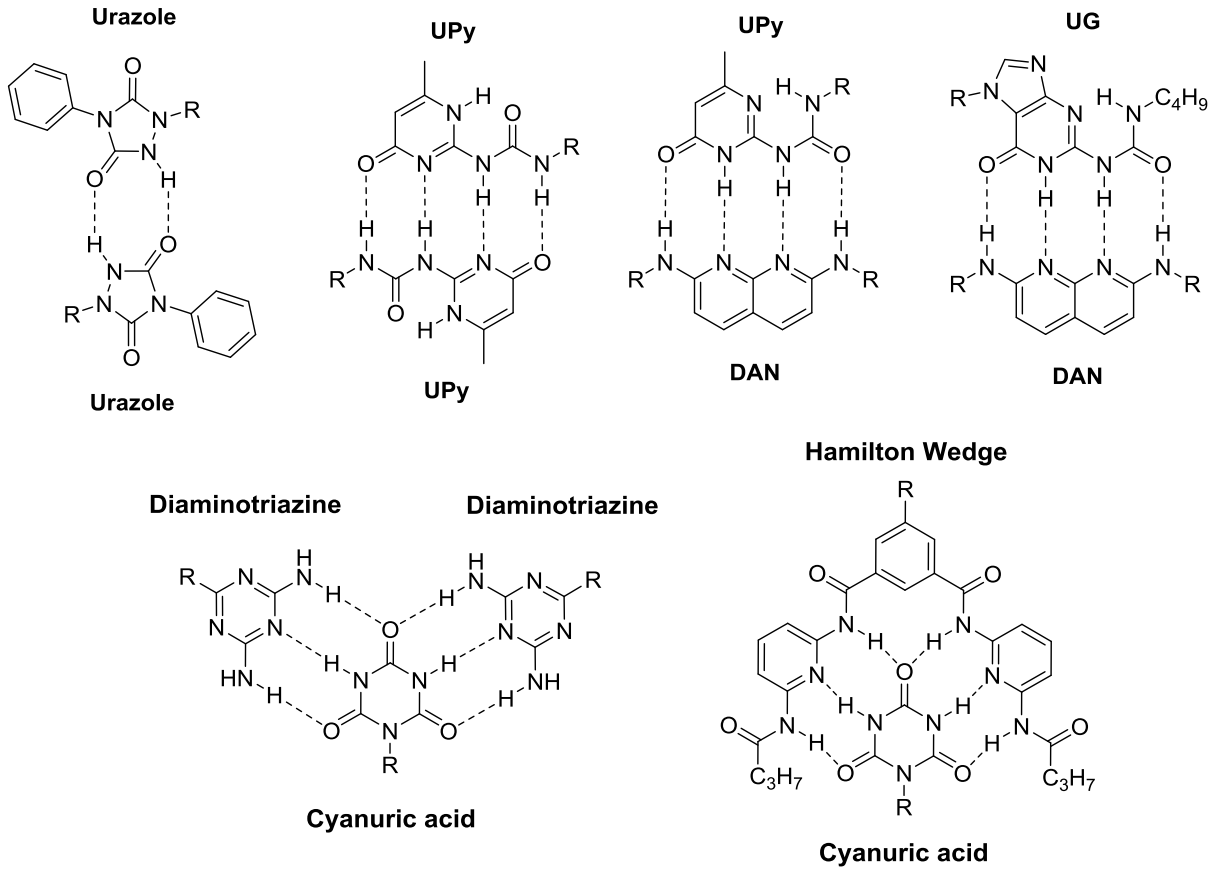


Figure 3. Impact of secondary interactions on the association constant of triple hydrogen bonded arrays. Green arrows indicate attractive secondary interactions, whereas red arrows indicate repulsive secondary interactions. Reprinted with permission from ref. 98. Copyright 2012 WILEY-VCH Verlag GmbH & Co KGaA.

Further calculations by Schneider and Sartorius show that these secondary interactions, either attractive or repulsive, contribute ±2.9 kJ mol^{-1} to the complex stability, whereas primary interactions contribute 7.9 kJ mol^{-1} for arrangements of several hydrogen bonds.[99] The binding strength of hydrogen bonded complexes can be increased by tautomerization, electronic substituent effects of the hydrogen bonding motifs, and preorganization.[100] For preorganization, often aromatic molecules are used as template structures, because they hamper the binding motifs ability to rotate around single covalent bonds; this allows for the presentation of all binding sites at once, thereby decreasing the overall entropy cost of the formation of hydrogen bonds.



Scheme 1. Hydrogen-bonding motifs discussed in this chapter.

In 1986, Stadler and coworkers reported on the first hydrogen bonded polymeric system; they used unpolar polybutadienes and functionalized them with urazole side groups that can form hydrogen bonds in a DA–AD fashion.[101] However, this double hydrogen bonded system was rather weak and even in the melt Newtonian flow was predominant under rheological probing at low frequencies. Despite the low association of the binding motif and the resulting material properties, Stadler could demonstrate the potential of the combination of hydrogen bonding motifs with polymers. However, it took more than ten years to develop a system that was stable enough to form supramolecular associates in organic solvents.

In 1997, Meijer and colleagues reported on the development of easily accessible derivatives of 2-ureido-4-pyrimidinone (UPy) with binding constants exceeding 10^6 M^{-1} in chloroform.[102] These quadruple hydrogen bonding motifs dimerize in a DDAA–AADD fashion and are preorganized by an intramolecular hydrogen bond.[91, 103] Meijer and coworkers utilized this motif by functionalizing polyethylene on its respective chain ends with UPy moieties.[104] They observed an increase of the viscosity in chloroform compared to solutions of unfunctionalized polyethylene, which was strongly concentration- and temperature-dependent. This observation indicates the formation of a supramolecular association of the chain ends with each other, leading to chain elongation of the polyethylene polymers. To prove this theory, a monofunctionalized polymer was added to inhibit this chain elongation, resulting in a decrease of the viscosity. On the basis of this research, Meijer and various other collaborators exploited this bonding motif and developed diverse ways to couple this motif to other functional polymers thereby allowing for the investigations of the network formation, network dynamics, and structure–property relations.[103,105–106] Moreover, this work also gave the

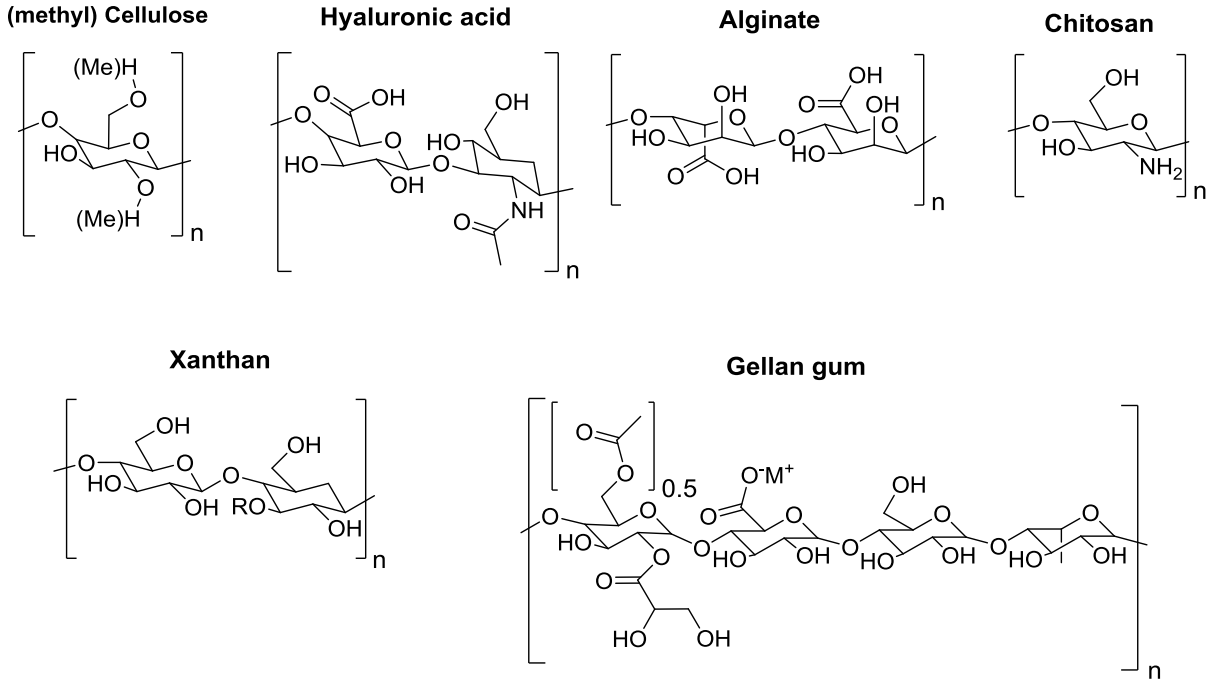
impulse for other researchers to explore hydrogen bonding as a basis for the development of supramolecular networks.[107-109]

Zimmermann and coworkers prepared a hetero-complementary hydrogen bonding motif based on 2,7-diamido-1,8-naphthyridine (DAN) that can form a quadruple DAAD hydrogen bonding array with a tautomer of UPy as well as with ureidoguanosine (UG), which are both ADDA motifs.[110–112] These binding motifs were then used to functionalize poly(butylmethacrylate) and polystyrene, respectively. In their native unfunctionalized form these two polymers are immiscible; however, dried films of DAN-functionalized polystyrene and UG-functionalized poly(butylmethacrylate) were transparent, indicating strong hydrogen bonding between the DAN and UG moieties ($K_{eq} > 10^8$ M^{-1} in chloroform). By contrast, the use of UPy-functionalized poly(butylmethacrylate) with DAN-functionalized polystyrene led to a viscous solution in chloroform, which exhibited lower values than the DAN–UG mixture in chloroform under rheological probing. Zimmerman and colleagues attributed this finding to the formation of self-complementary hydrogen bonding of the UPy moieties as a side reaction.

Weck and coworkers introduced an approach to form supramolecular networks by copolymerizing cyanuric acid functionalized norbornene monomers as well as unfunctionalized spacer monomers in a ring-opening metathesis polymerization.[113] Upon addition of a bivalent low molecular crosslinker based on 2,4-diaminotriazine, which can associate to the cyanuric acid via triple hydrogen bonding interaction, highly viscoelastic gels are formed in 1-chloronapthalene. This effect is attributed to the formation of a six-point hydrogen bonding array of two diaminotriazine crosslinkers to one cyanuric acid moiety, leading to a high network connectivity and a long lifetime of the supramolecular association. Additionally, Weck and colleagues prepared another bivalent low molecular crosslinker, a so-called Hamilton wedge, which can form six-point hydrogen bonding with cyanuric acid; surprisingly, after addition of this more sophisticated crosslinker to the cyanuric acid functionalized polymers only highly viscous fluids could be observed. As a result, macroscopic mechanical properties of supramolecular networks are not only dependent on the strength of the hydrogen bonding, but are also influenced by the assembly of the crosslinks and their respective microstructures. This effect has also been observed on low molecular supramolecular aggregates, which can form larger and more stable aggregates upon stacking and clustering of the associated supramolecular crosslinks.[114–115]

Even though the aforementioned examples have been the sound basis for gaining understanding of supramolecular networks, they are not suitable for any applications. The main reason for that is the choice of organic solvents. For most applications either water is needed to be used or no solvent at all. However, water can act as a donor as well as an acceptor for hydrogen bonding motifs, impeding the desired formation of hydrogen bonds and thereby strongly weakening the resulting material properties. To overcome this limitation, in the last century natural polysaccharides, such as cellulose,[116–118] agarose,[119–121] and starch,[122–124] or composite materials of either natural polymers or natural polymers and synthetic polymers have been used. In these natural polymers water also acts as a competitor towards hydrogen bonding motifs, but the higher number of functional groups in close proximity to each other in comparison to synthetic polymers renders this side reaction rather unlikely. Thus, in their native state some of these biopolymers are not water soluble at room temperature and thereby require chemical modification prior to be used in any application. This can be achieved by partly alkylation of hydroxy moieties present in these polysaccharides.[125-126] In the case of cellulose, the most abundant alkylated derivatives are methyl, ethyl, hydroxyethyl, and hydroxypropylmethyl cellulose. These derivatives are all water soluble at

room temperature and form hydrogels upon temperature increase. The sol–gel transition can be described by the formation of hydrophobic alkylated domains in the network at elevated temperatures, entailing a repulse of water in these domains and the subsequent formation of hydrogels. This network formation can also be triggered by the addition of salts; the solvation of salts is a competing reaction to the hydrogen bonding between water molecules and the polysaccharides, allowing for the formation of physical networks even at room temperature.



Scheme 2. Overview of polysaccharides discussed in this thesis. The rest R in the xanthan structure consists of a trisaccharide (mannose–glucuronic acid–mannose acetyl).

Alkylated polysaccharide derivatives have applications in our daily lives as thickening agents in food industry, emulsion stabilizers in cosmetics, packaging materials, as well as humectants in pharmacy. In the last 20 years, several projects focused on the utilization of these biopolymers, in either their native or modified form, for applications in the medical field. However, hydrogels that are based on these hydrogen bonded biopolymers were degraded too fast to allow for any specific application. To overcome this limitation, composite materials, such as blends of cellulose with polyvinyl alcohol,[127–128] hyaluronic acid,[117,129] chitin,[130–131] or chitosan[131] have been developed.

Shoichet and coworkers reported on a system based on methylcellulose and hyaluronic acid for implantation and subsequent drug or cell release.[129] Their working hypothesis was to design a material platform that can form gels suitably fast to avoid spreading after implantation, that can be implanted minimally invasively, and that avoids immune reactions. Testing of several methylcellulose compositions, e.g., a blend of hyaluronic acid and methylcellulose and an acetylated version of this blend, towards their mechanical properties, degradation profiles, cell adhesive properties, and their in-vivo immune response, revealed that a blend of 2% hyaluronic acid and 7% methylcellulose met all the criteria of the initial working hypothesis. This hydrogel allows for implantation via syringe and degrades on a precisely tunable timescale of 1 to 28 days. Additionally, this material can be loaded with active agents, rendering it useful for diffusion-limited and particle-mediated drug delivery. In more recent publications, Shoichet and colleagues investigated the utilization of this material

towards cell transplantation and drug delivery, thereby broadening the applicability of this system for treatment of injuries in the spinal cord.[132–133]

Zhang and coworkers prepared hydrogels based on poly(vinyl alcohol)/cellulose blends by chemical as well as physical crosslinking; chemical crosslinking was achieved by addition of epichlorohydrin to the polymer blend, thereby covalently crosslinking the hydroxy moieties of the polymers. Repeated freeze and thaw cycles of an aqueous solution of the blend was used to prepare the physically crosslinked analogue. Both polymer blends were then investigated towards their structure–property relationship. The physically crosslinked blend exhibited high mechanical strength, but a low swelling ratio, indicating a dense microstructure between the single polymer chains. By contrast, the chemically crosslinked hydrogels exhibited a high swelling ratio and low mechanical strength, indicating a porous microstructure.[134]

The first fully *synthetic* hydrogen bonded hydrogel has been recently reported by Meijer and coworkers.[135] This system is based on poly(ethylene glycol) (PEG), which is end-group functionalized with UPy moieties; additionally, hydrophobic alkyl spacer chains were placed in between the UPy moieties and the polymer chain. Hydrogels can be prepared by dissolving dried polymers in isotonic water at 70 °C and cooling down the mixture to room temperature. The proposed mechanism of hydrogel formation is summarized in Figure 4A. In dilute solution, the polymer chains aggregate to form isolated nanofibers by lateral hydrogen bonding of urea motifs present in the hydrophobic spacer chains. Upon increase of the polymer concentration, these nanofibers form a transient network.

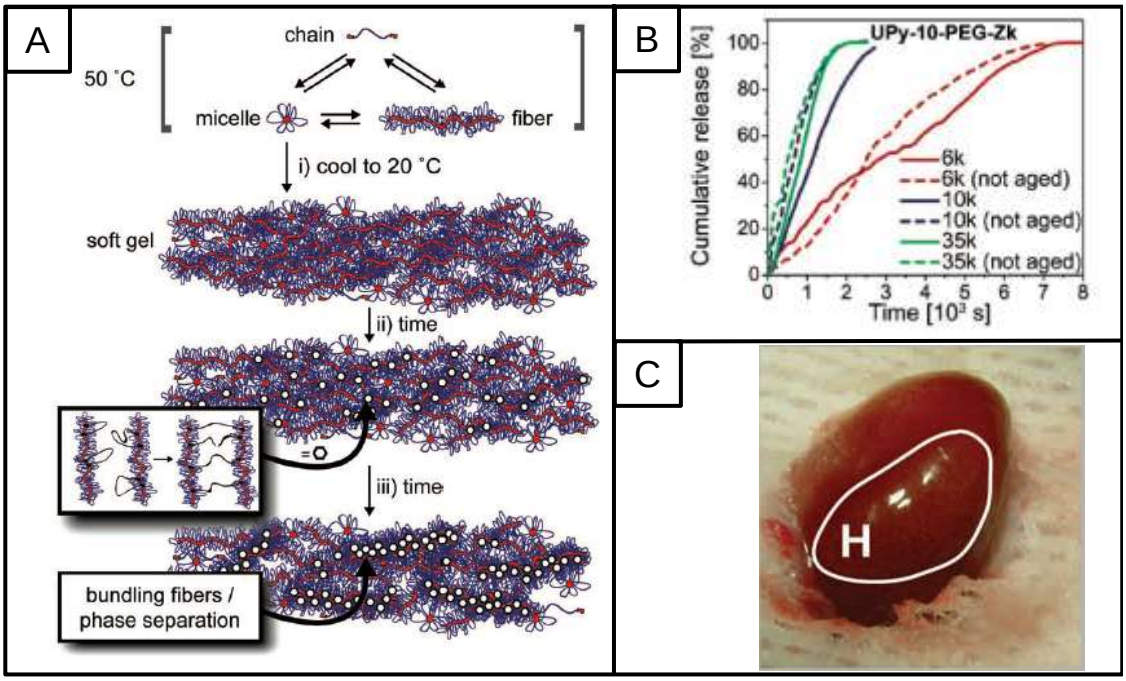


Figure 4. Formation and characterization of hydrogels based on poly(ethylene glycol) (PEG) chains that are endgroup-functionalized with ureidopyrimidinone (UPy). (A) The process of hydrogel formation of UPy-modified PEG by assembly of different structural units at 50 °C, involving single, micelles, and fibers. (i) Upon cooling or increase of the concentration, a soft hydrogel forms. (ii) Formation of supramolecular crosslinks after 16–24 h. (iii) Bundling of fibers, leading to phase-separating domains. (B) Rhodamine B release from aged and freshly prepared hydrogels depending on the chain length of the polymers used to prepare the hydrogels (red PEG 6 kDa, blue PEG 10 kDa, green PEG 35 kDa). (C) Implantation of the hydrogel into a kidney. The whitely marked area indicates

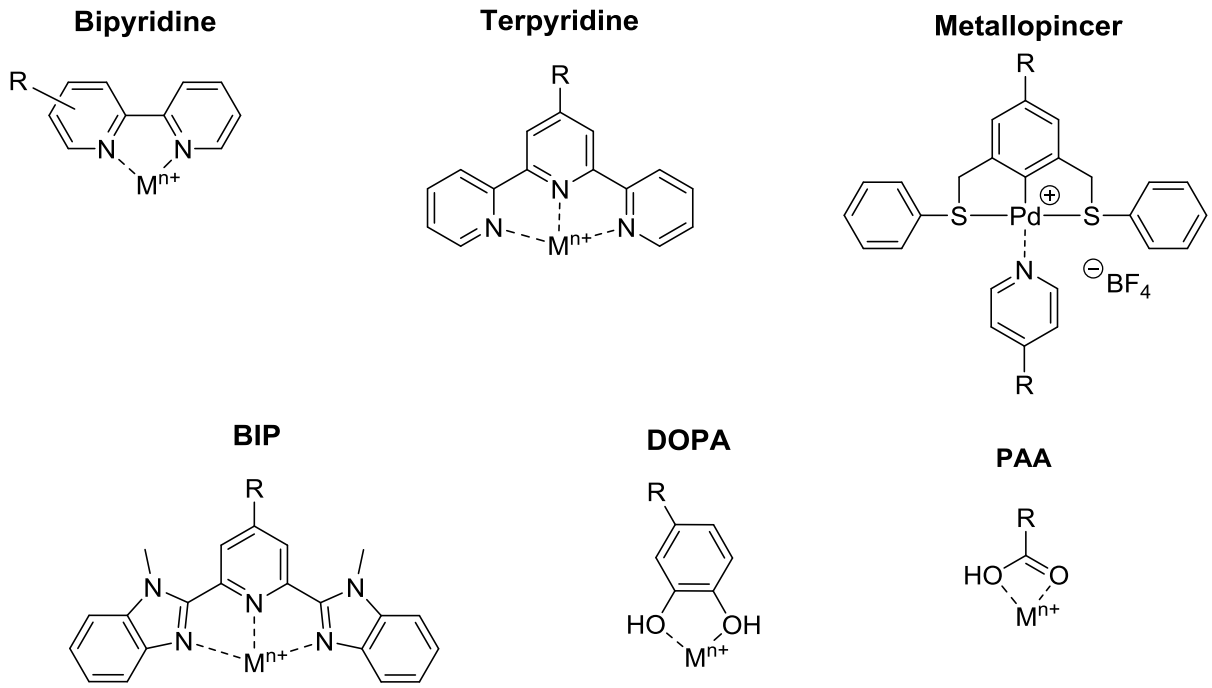
where the hydrogel (H) is located. Modified with permission from ref. 135. Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA.

Meijer and collaborators observed an increase in the mechanical properties after 16–24h, which they attribute to the formation of additional intramolecular crosslinks. The equilibrium mechanical properties can be tuned by the length of the hydrophobic alkyl spacer and the poly(ethylene glycol) chain length. To investigate the erosion of the hydrogels, rhodamine B-loaded gels were prepared and investigated in a flow chamber under a confocal microscope. Hydrogels formed from short polymer (6 kDa) chains released the dye six times slower than hydrogels composed of long chains (35 kDa). In addition, aged samples released the dye even slower than their freshly prepared equivalents, as summarized in Figure 4B. The same experiment was repeated with hydrogels loaded with a fluorescently tagged protein, showing a complete reversed result; here, hydrogels composed of longer polymer chains exhibited a slower release of the protein. When longer alkyl spacer chains were used, the release of the protein could be prolonged further. With these results in hand, Meijer and colleagues prepared another set of hydrogels containing a bone morphogenetic protein and injected it into kidneys of rats, as shown in Figure 4C. Seven days after implantation, the kidneys were investigated by histology and no immune response could be observed towards the completely eroded hydrogels.

In a follow-up publication, Meijer and coworkers investigated the rheological properties of these materials in much more detail and their application as a drug delivery system for the renal region.[136–138]

1.1.2. Metal Complexation

Another widely applied supramolecular interaction to build up linear supramolecular polymers, gels, and networks, is metal complexation; here, metal ions are complexed by suitable ligand-moieties attached to polymer chains, resulting in binding energies of up to 400 kJ mol^{-1} depending on the metal-ligand system used.[139] Because of these high binding energies and the possibility to use many different metal ions in various oxidation states, a variety of different ligands have been developed. As a result, supramolecular assemblies have been reported that exhibit stabilities ranging from very labile to covalent-like. The most prominent and therefore most extensively studied ligand systems are bi- and terpyridines.[22,140] Bipyridines coordinate to metal(II)-ions in a trivalent fashion, whereas terpyridines coordinate to metal(II)-ions in a bivalent manner.



Scheme 3. Overview of metal-complexes discussed in this thesis.

In a seminal work, Schubert and colleagues used terpyridine-functionalized methacrylate and methyl methacrylate monomers to prepare copolymers that can be crosslinked by metal complexation.[141] Addition of iron(II) and zinc(II) ions to the copolymers in a chloroform/methanol mixture at low concentrations (17–40 g L^{-1}) led to formation of supramolecular assemblies that were studied by UV–vis spectroscopy and viscosity measurements. Schubert and collaborators observed a characteristic UV–vis absorption band at 558 nm in the presence of metal ions, which is attributed to a charge transfer from the metal ion to the terpyridine ligand. After addition of the metal ions, the solutions showed an increase of their respective viscosities. This effect was more distinct in the case of the iron(II) ions, due to the stronger iron–terpyridine interaction. To further investigate the differences in the binding strength, the complexed solutions were dried and redissolved, leading to a gel-like appearance of the iron-complexed polymer and a clear solution in the case of the zinc-complexed polymer. Lastly, the solutions were treated with hydroxyethyl ethylenediaminetriacetic acid (HEEDTA), which is a strong chelating ligand and therefore acts as a competitive ligand to terpyridine. Addition of HEEDTA to the iron(II) complexes led to the discoloration and a decrease of the viscosity of the solution, demonstrating the reversibility of the metal complexation and thereby the stimuli-responsiveness of the material.

In another approach, Schubert and coworkers functionalized commercially available poly(vinyl chloride) (PVC) with terpyridine moieties via a post-polymerization procedure.[142] In contrast to the copolymerization of functional monomers, post-polymerization functionalization allows for a better control of the degree of functional groups; however, more effort is needed to prepare these materials. In a first step, PVC was activated with (2-mercapto-phenyl)-methanol to introduce hydroxyl groups to the polymer backbone. In the second step, isocyanate-functionalized terpyridine was added to the activated PVC, yielding in terpyridine-functionalized PVC. This material was then investigated towards its capability to form supramolecular assemblies using Cd(II), Co(II), Fe(II), Mn(II), Ni(II), and Zn(II) ions, showing characteristic absorptions in UV–vis spectroscopy in chloroform. Additionally, Schubert and colleagues could show that grafting of a small molecule to the PVC backbone can also be realized by metal complexation. To achieve this, a methyl diethylene

glycol-functionalized terpyridine was treated with equimolar amounts of $RuCl_3$, yielding in a monocomplex, which was then added to the functionalized PVC. Subsequent size exclusion chromatography (SEC) showed a shift to a faster retention time compared to unmodified and terpyridine-functionalized PVC, which indicates the presence of a polymeric material with a higher molecular mass. Additionally, UV–vis spectra of the material showed a characteristic metal-to-ligand-charge-transfer band of the Ru(II)–bisterpyridine complex at 490 nm, demonstrating the possibility to employ supramolecular interactions as tools for grafting small molecules to functional high-molecular-weight polymers. In more recent work, Schubert and colleagues exploited terpyridine-crosslinked polymers further by developing microwave-assisted postpolymerization functionalizations,[143] complex supramolecular polymers that possess optoelectronic properties,[144] as well as stimuli-responsive micelles.[145]

Another prominent class of ligands are bipyridines. One of the first polymer functionalizations with bipyridines was conducted by Card and Neckers as early as 1977.[146] Even though the aim of this work was to develop a system that is capable to determine metal contents in organic solutions as well as act as a catalyst, this can be considered one of the first works on metallo-supramolecular polymer networks in organic solvents. In their approach, Card and Neckers employed electrophilic aromatic substitution to brominate and subsequently lithiate commercially available polystyrene (PS) beads before adding bipyridine to complete the functionalization. Then, the product was investigated towards its swelling factor in various solvents such as methanol, acetonitrile, ethyl acetate, tetrahydrofuran (THF), and benzene. In general, the functionalized PS beads swelled much less in comparison to the unfunctionalized polymer beads; this finding was attributed to unwanted crosslinking side reactions during the functionalization or expansion of the polymer beads caused by the incorporation of bipyridines. After addition of transition metal ions, such as Cr(III), Mn(II), Fe(II), Fe(III), Co(II), Ni(II), Pd(II), and Cu(II), to the polymer beads in THF, the amount of incorporated metal complexes was determined by elemental analysis, IR, and UV–vis spectroscopy. Even though the analytical methods were in agreement about the amount of metal incorporation of each individual transition metal ion, every metal salt was incorporated in different quantities, with Fe(III) ions showing the highest quantity. To determine the sensitivity of polymer beads toward metal complexation, Fe(III) containing solutions of different concentrations were added to the polymer beads in THF, showing a quantitative complexation of the metal ions, even for concentrations as low as 10^{-7} M. Lastly, Card and Neckers investigated the effect of the solvent on the beads by repeating the swelling experiments with Fe(III) ions present. For nonswelling solvents almost no metal incorporation was observed, whereas in good solvents a complete complexation of the bipyridine ligands was observed. The authors attributed this finding to the ligands being inside the beads in nonswelling solvents, whereas upon swelling of the beads the ligands can be attained by the Fe(III) ions and complexation occurs.

In another approach, Nozakura and coworkers prepared 4-methyl-4'-vinylbipyridine and 6-vinylbipyridine and their respective homopolymers by free radical polymerization to photochemically convert solar energy with the help of tris(bipyridine)ruthenium(II) complexes.[147] The homopolymer prepared from 4-methyl-4'-vinylbipyridine was insoluble due to extensive crosslinking side reactions during the preparation, whereas the homopolymer prepared from 6-vinylbipyridine was soluble in organic solvents. Interestingly, the properties of the polymers changed after addition of Ru(II) salts; the solubility of poly(6-vinylbipyridine) in organic solvents decreased, while the solubility in water increased. The addition of Ru(II) ions to crosslinked poly(4methyl-4'vinylbipyridine) resulted in a voluminous gel. In a follow-up study, Nozakura and colleagues presented an alternative synthetic

route to the preparation of 4-methyl-4'-vinylbipyridine, resulting in a homopolymer that is soluble in common organic solvents. Moreover, the photophysical properties of such polymer networks with pendant tris(bipyridine)ruthenium(II) complexes were studied in more detail.[148]

In a more recent work, Weder and coworkers functionalized poly(*p*-phenylene ethynylene) (PPE) with bipyridines in the main chain to obtain conjugated supramolecularly crosslinked polymer networks with optoelectronic properties.[149] Upon addition of transition metal ions (Cd(II), Co(II), Cu(I), Ni(II), or Zn(II)) the bipyridine-functionalized polymer chains formed three-dimensional networks in mixtures of chloroform and acetonitrile. Polymer networks crosslinked by transition metals with a d^{10} electron configuration, such as Zn(II) and Cd(II), are light emissive, while the networks crosslinked by Co(II), Cu(I), and Zn(II) ions possess nonradiative metal-to-ligand charge-transfer complexes.

In a similar approach to their investigation of hydrogen-bonded supramolecular polymer gels, Weck and coworkers prepared side-chain functionalized polynorbornenes, which contain both hydrogen-bonding motifs and palladated metallopincer complexes.[94,150] Upon addition of a small molecular crosslinker based on pyridine, metallo-supramolecular crosslinking occurs by further complexation to the Pd central atoms. For the hydrogen-bonding, cyanuric acid moieties were incorporated in the side-chain and crosslinking occurred by addition of small molecular diaminotriazine derivatives. This design principle allows for orthogonal polymer crosslinking by addition of suitable small molecular crosslinkers to the polymer solutions in chloroform. Polymer networks crosslinked solely by hydrogen bonding showed only a slight increase of the viscosity, whereas metallo-supramolecular networks exhibited a drastic increase in their viscosity. Elaborating on their work, Weck and collaborators investigated the orthogonal decrosslinking of polymer networks, which contain both metal-complexation and hydrogen-bonding sites, in 1-chloronapthalene using similarly functionalized polynorbornenes.[94] To achieve this, supramolecularly crosslinked polymer gels were treated with a monotopic end-capping agent to disrupt hydrogen-bonding without interfering with the metal complexation or by simply heating the polymer gel, which again affects only the hydrogen-bonding. The metal-coordinated crosslinks are chemo-responsive and can thereby be affected by the addition of a ligand displacement agent, in this case triphenylphosphine. By this means, multi-responsive organogels can be prepared from a single polymer backbone and the mechanical properties of such a material can be tuned by selective crosslinking or de-crosslinking the polymer network.

The previous examples of metallo-supramolecular polymer networks utilized transition metal ions as central atoms in the complexes. Crosslinking occurred by using bivalent or trivalent ligands in either the side-chain of polymers or as functional end groups of polymer chains. Exploiting the ability of lanthanide metals to form complexes with up to three *tri*dentate ligands, Beck and Rowan prepared multi-responsive polymer gels from linear bifunctional macromonomers.[151] To achieve this, an oligo-PEG chain was end group functionalized with 2,6-bis(1-methylbenzimidazolyl)pyridine (BIP) on both chain ends and after addition of either La(III) and Co(II) or Eu(III) and Zn(II) to the functional macromonomers, gels were formed in a mixture of chloroform and acetonitrile. The transition metal ions promote linear chain extension, whereas the lanthanide metals act as crosslinking agents, as depicted in Figure 5A. By combining these metals, Beck and Rowan were able to prepare a set of four different polymer gels crosslinked by Co/La, Co/Eu, Zn/La, and Zn/Eu. Then, the gels were dried and reswollen in acetonitrile, whereupon the gels exhibited thermo-responsive properties. At 100 °C, the Co/La gel showed a reversible sol–gel transition. Upon further increase of the temperature, the yellow color of the polymer solution persisted, which indicates that only the

lanthanum complex was disrupted, whereas the yellow colored Co complex was still present. Shaking of the gels resulted in free-flowing liquids, which upon standing for several seconds reformed gel-like materials, as shown in Figure 5B. This thixotropic effect depends on the amount of solvent present in the gels; gels with less solvent recovered more quickly. Furthermore, the crosslinking could be disrupted by addition of carboxylic acids. This chemo-responsiveness is caused by the tendency of lanthanides to form complexes with such acids, thereby dispersing the BIP-complexes. In several follow-up studies, Rowan and coworkers explored these systems further by investigating the influence of different types of metal and counter ions, the gelation mechanism, and network reconstruction.[152–156]

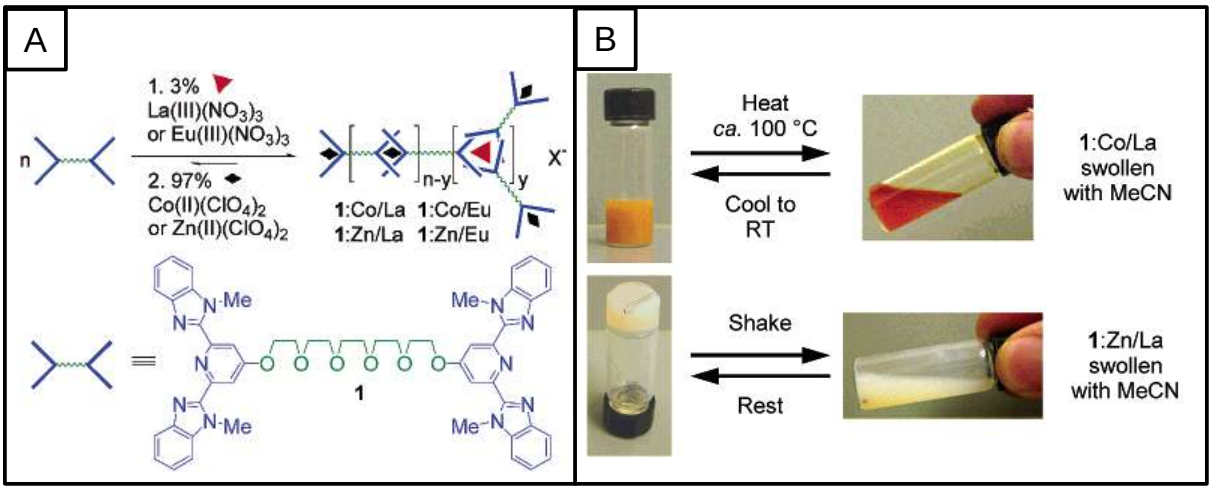


Figure 5. Metallo-supramolecular organogels as introduced by Beck and Rowan. (A) Polymer networks are formed by complexation of lanthanide and transition metal ions to 2,6-bis(1-methylbenzimidazolyl)pyridine (BIP) functionalized poly(ethylene glycol) in mixtures of chloroform and acetonitrile. (B) Thermo- and mechano-responsiveness of these gels. Reprinted with permission from ref. 151. Copyright 2003 American Chemical Society.

In contrast to hydrogen-bonded polymer gels, hydrogels are not that rare among metallo-supramolecular polymer gels. Nozakura and coworkers were already able to prepare hydrogels in their exploration of ruthenium-based polymer gels to utilize solar energy in the early 1980's.[147–148] However, the use of toxic or catalytically active metals limits the use of such gels to applications like chemosensors,[22] electronics,[157] and solar energy conversion.[147–148] When using metals that are already present in biological systems or are biologically inert, such as Mg, Ca, Fe, Zn, Pt, or Au, these hydrogels can be used for more demanding life science or medical applications. The most prominent polymeric material based on metal complexation in life science applications is alginate. This polysaccharide is obtained from brown algae and is composed of guluronic and mannuronic acid blocks or sequences in three different fashions: blocks of guluronic acid, blocks of mannuronic acid, and alternating sequences of both. Gelation occurs upon addition of Mg^{2+}, Ca^{2+}, or Ba^{2+} ions by complexation to the carboxyl groups of the sugar units. The mechanical properties of the resulting hydrogels strongly depend on two factors: the ratio of guluronic to mannuronic acid and the concentration of the metal ions added. Because of their biocompatibility, and their bio-inert and non-immunogenic properties, alginates are used as wound dressings, for dental impression, and as extracellular matrixes for tissue engineering. When used as a wound dressing, the hydrogels exchange Ca^{2+} ions with Na^{+} ions upon contact with exudate, leading to an uncontrolled decrosslinking of the hydrogel, which releases water and keeps the wound moist. Whereas this effect

supports the healing of topical wounds, for in vivo applications, controlled hydrogel degradation is preferred. To achieve this, Mooney and coworkers partially oxidized alginates to alter their degradation profile.[158] This modification renders the treated alginates susceptible towards hydrolysis. To investigate their biocompatibility, myoblast cells were seeded on top of modified alginates and native alginates, showing no difference between the two types of hydrogels. Recent research is also focused on the preparation of alginate hydrogels using other metal ions, on alginate modifications to allow for mammalian cell attachment, and on alginates as vessels for drug delivery.[49]

A common method in the development or improvement of synthetic materials is to mimic Nature. Mostly, favorable properties are mimicked, such as bone structures for lightweight but robust materials or the self-cleaning properties of lotus plants. An undesired effect has been investigated quite extensively by the groups of Messersmith and Waite: the adhesive properties of mussels.[34,159–163] The mussels possess byssal threads that allow them to physically adhere to surfaces, e.g., the hulls of ships, which greatly increase the operating and support costs of naval fleets worldwide. On the surface of these threads, catechol units are present, which can form complexes with Fe(III) ions. Depending on the surrounding pH, either weak monocatechol complexes (pH ≤ 5) or bis- and tris-complexes (pH ≥ 8) are formed, as visualized in Figure 6A. The latter complexes possess some of the highest known supramolecular stability constants with K_{eq} up to 10^{40} M^{-1}.[34] The force needed to rupture such a complex is modestly lower than the force needed to rupture a covalent bond; however, the metallo-supramolecular network can self-heal, whereas the covalent bond remains cleaved. Messersmith and Waite prepared hydrogels based on tetra-arm PEG functionalized with dihydroxy-phenylalanine (DOPA), a catechol-like amino acid.[161] Upon addition of Fe(III) ions to the functionalized polymer, a blue-green fluid was obtained at pH 5. After increasing the pH to 8, a sticky purple gel was formed and at pH 12, a red elastomeric gel was obtained, as compiled in Figure 6B. UV–vis and Raman spectroscopy could show that higher-ordered complexes are formed upon increase of pH. To compare the mechanical properties of these hydrogels to a covalently crosslinked hydrogel, the latter one was prepared by addition of sodium periodate to DOPA-functionalized PEG, thereby chemically crosslinking the DOPA moieties. Both hydrogels showed almost the same elastic modulus at high frequencies; however, after applying a high strain to the gels, the chemically crosslinked gel was irreversibly damaged, whereas the physical gel recovered its elastic modulus within minutes. Addition of ethylenediaminetetraacetic acid (EDTA), a chelating agent, to the metallo-supramolecular hydrogels led to the degradation of the hydrogels within an hour. In a recent work, Waite and coworkers employed a DOPA derivative, 3-hydroxy-4-pyridinone, to functionalize again tetra-arm PEG and subsequently form hydrogels by addition of Fe(III) ions. This system is capable to form hydrogels at physiological pH and gelation can be achieved with bio-relevant metal ions, such as Al(III), Ga(III), and Cu(II), allowing for the tuning of the degradation profile for controlled release applications.

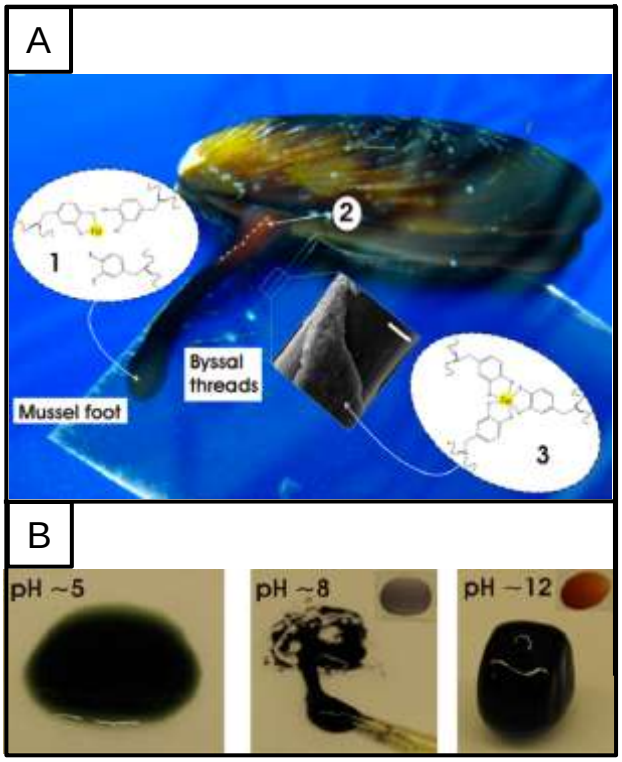


Figure 6. Mussel-inspired DOPA-Fe(III) crosslinking. (A) Schematic of mussels adhering to surfaces by formation of Fe(III)-catechol complexes. (1) Production and storage of byssal thread in epithelium cells at pH 5 through monocatechol complexes in the mussel foot. (2) Release of the threads from the ventral grove as indicated by the dashed white line. (3) Formation of bis- and triscatechol Fe(III) complexes after exposure to slightly basic (pH 8) water. (B) Polymer networks formed by complexation of dihydroxy-phenylalanine (DOPA) attached to poly(ethylene glycol) with Fe(III) ions at various pH values. Reprinted with permission from ref. 161. Copyright 2011 National Academy of Sciences of the United States of America.

In another approach, Seiffert and coworkers employed bipyridine-functionalized linear PEG to prepare cell-laden microgels.[75] For this purpose, commercially available PEG was converted to PEG-diamine and the functionalization was completed by amide-coupling of bipyridine-COOH to the polymer termini. Upon addition of Fe(II) ions to the functionalized PEG, metallo-supramolecular polymer networks were obtained. Combining the technique of droplet-based microfluidics and supramolecular chain crosslinking, the authors were able to prepare microgels whose elasticity was controlled by the PEG chain length as well as the polymer concentration used, as shown in Figure 7A. To investigate the biocompatibility of this material, mammalian cells were encapsulated into microgels with different elasticities and the cell viabilities were determined, respectively, as compiled in Figure 7B. Cell viabilities exceeding 90% could be obtained for both suspension and adherent cells by using a microgel composition with an elastic modulus of 4.8 kPa employing a PEG 6 kDa backbone at a concentration of 133 g L^{-1}. The use of an increased precursor concentration or of a PEG 1.5 kDa backbone negatively influenced the cell viabilities of suspension cells, whereas adherent cells exhibited increased viabilities at higher concentrations of 267 g L^{-1} using a functionalized PEG 6 kDa backbone. Addition of the chelating agent EDTA to the cell-laden microgels disrupted the metallo-supramolecular crosslinks, thereby releasing the cells without affecting the cell viabilities. With this work, the authors developed a material platform that potentially allows for the study, storage, and manipulation of cells in an extracellular matrix with the option of a subsequent release of such cells.

Furthermore, this design principle allows for the assembly of tailor-made tissues from microgel building blocks.

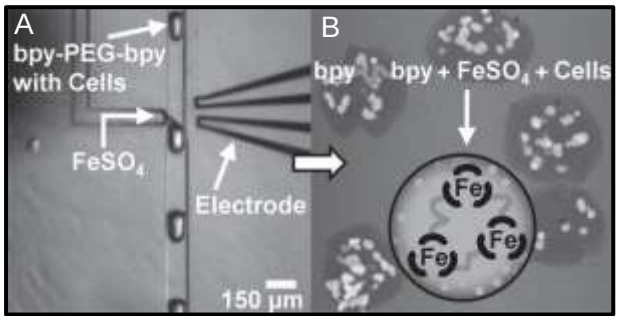


Figure 7. Droplet-based fabrication of cell-laden microgel particles. (A) Premicrogel droplets containing crosslinkable bipyridine-functionalized poly(ethylene glycol) and cells are injected with Fe(II) by using a picoinjection unit. (B) Microgel particles crosslinked by Fe(II) ions containing living cells. Reprinted with permission from ref. 75. Copyright 2013 WILEY-VCH Verlag GmbH & Co KGaA.

In a recent work, Karperien and coworkers employed eight-arm PEG and functionalized five of the eight terminal hydroxy groups with terpyridine to prepare biocompatible supramolecularly crosslinked hydrogels.[164] Interestingly, depending on the polymer concentration either nanogels with an average diameter of 250 nm or macroscopic hydrogels were obtained upon addition of Fe(II) ions. The authors attributed the formation of the nanoparticles to the tendency of the terpyridine-functionalized PEG to form micelles of ~10 nm in water at low concentrations and to the assembly of such micelles into larger aggregates upon addition of metal ions. This hypothesis has been verified by scanning electron microscopy (SEM), dynamic light scattering (DLS), and ^{1}H-NMR experiments. Furthermore, increase of the polymer concentrations resulted in an increase of the elastic moduli of the macroscopic hydrogels. To investigate the cytotoxicity of terpyridine-functionalized PEG, both with and without Fe(II) ions, bovine chondrocytes were used. Interestingly, a high cytotoxicity of the uncomplexed functional polymer was observed. This was attributed to the depletion of essential metals from the cells by the uncomplexed terpyridine moieties. However, the hydrogels showed no cytotoxicity at all. To exclude potential leaching of cytotoxic compounds from the hydrogels, a transwells system was used, which connects both cells and hydrogels by a semipermeable membrane, thereby allowing for the diffusion of small molecules. After seven days, no significant difference of the cell viability compared to a cell culture of the same age was observed. This study showed the potential of partially terpyridine-functionalized PEG for biomedical and/or pharmaceutical applications.

1.1.3. Ionic Interactions

Ionic interactions between oppositely charged functional moieties attached to polymer chains provide an alternative to covalent crosslinking.[165–166] The resulting polymer gels are extremely robust materials, but can easily be degraded by changes of the pH or the salt concentration at the same time. Ionic polymer gels can be obtained by either combining polymers of opposite charge or by addition of suitably charged small molecules to functional polymers. However, mixing of oppositely charged polymers is often impossible, due to macroscopic phase separation.[40] To overcome this limitation, polyelectrolytes based on block copolymers are used. A common design principle for such a block copolymer is the use of a soluble midblock and charged endblocks. In

solution, micelles consisting of discrete solvophobic domains and a solvophilic corona are formed by microscopic phase separation of the insoluble blocks. Gel formation occurs by interconnection of these hydrophobic crosslinking domains by the solvophilic coronas.[40,166] In contrast to polymer networks that are crosslinked by either hydrogen bonding or metal complexation, organogels are rather rarely crosslinked ionically, due to solubility issues of charged molecules in organic solvents. One example for organogels has been introduced by Zhang and Guo by using ABA triblock and AB diblock polymers.[167] The triblock consisted of two acidic sulfonated polystyrene endblocks and a neutral solvophilic poly(ethylene-*ran*-butylene) midblock, whereas the oppositely charged AB diblock consisted of a solvophilic polystyrene block and a basic poly(2-vinylpyridine) block. Gelation occurred by mixing a solution of the triblock in a toluene/methanol mixture and a solution of the diblock in toluene. The acid-base reaction of the sulfonate and pyridine moieties has a high reaction rate and therefore gelation occurred within 20–30 seconds without phase separation, resulting in clear organogels. Rheological probing of gels with different compositions showed that the pyridine content in the gels has an influence on the mechanical properties of the gels. This is due to the formation of denser polymer networks with increasing pyridine content. To investigate this finding in more detail, Zhang and Guo dried the gels and probed them by FT-IR spectroscopy, showing that even at low pyridine concentrations uncomplexed pyridines are present in the gels. Upon increase of the pyridine concentration, the characteristic complex bands are getting more distinct, indicating the formation of more ionic complexes, which is in agreement with the rheological data. Small angle X-ray scattering showed that on average ten sulfonated polystyrene blocks react with one pyridine block and that the solvophilic blocks form shells connecting and stabilizing the solvophobic cores. Upon addition of organic acids, bases, and salts to the organogels, the mechanical properties of the gels can be tuned from highly viscoelastic to free flowing. In a follow-up study, Zhang and Guo employed this system for the preparation of high internal phase emulsions, which can potentially be used as templates for porous materials and as separation membranes.[168]

For biomedical applications hydrogels are preferred. An alternative to the time-consuming preparation of synthetic block copolymers is the use of functional biopolymers; a well-investigated example is chitosan,[46,169–171] a polysaccharide composed of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. Chitosan is a derivative of chitin, the main component of the exoskeletons of crustaceans and insects, and is therefore non-toxic, biocompatible, biodegradable, and has a high natural abundance in form of chitin. Hydrogels can be obtained by mixing of chitosan with polyanions, including xanthan, hyaluronic acid, alginate, collagen, pectin, and gelatin and thus has raised interests for applications, such as topical ocular drug delivery,[46] implantation,[171] wound healing,[172] and cosmetics.[173]

In 1994, Dimitriu and coworkers reported on a hydrogel composed of chitosan and xanthan, a polyanionic polysaccharide that is also produced on industrial scale.[174] Investigation of the network structure by electron microscopy showed porous fibrillar structures, rendering this system interesting for drug delivery and cell encapsulation. In an effort to investigate the structure-property relationship of the hydrogels, Dimitriu and colleagues varied the precursor polymer composition and found the gelation to be based on a coacervation mechanism. The mechanical properties of the resulting hydrogels can be tuned by aging of the hydrogels, due to formation of denser polymer networks with rising time. Additionally, aged hydrogels that gained a denser network structure have less distinct swelling properties than freshly prepared ones. Interestingly, in strongly acidic, pH ≤ 2, and in weakly acidic to very basic media, pH ≥ 5.8, the swelling degree of the hydrogels is at its highest, due to the disruption of the ionic crosslinking. Following this study, Dimitriu and

collaborators broadened the knowledge about this system and improved the materials properties for applications ranging from immobilization of enzymes to dermatology.[69,165,175–180]

In another approach, Gander and coworkers employed hydrogels composed of chitosan and alginate to prepare neural conduits.[181] To achieve this, tubular hydrogels were prepared using a spinning mandrel and their network structure was investigated by SEM, showing a highly porous microstructure of the conduits. Then, the diffusivity of the material was investigated using fluorescein-labeled dextrans of different molecular weights that were filled inside these tubular hydrogels and the amount of leaked dextrans was determined fluorometrically. The dextran with the highest molecular weight (20 kDa) showed almost no diffusivity out of the hydrogels, whereas 35% of the low molecular weight dextrans (4 kDa) were leaked within seven hours. This experiment served as a simulation for the diffusivity of nutrients and metabolites, showing that no high-molecular proteins, cells, or immunoglobulins can permeate the conduit wall, which could trigger an immune reaction and thereby negatively affecting the regeneration of the neural tissue. Lastly, Gander and collaborators investigated the Young's modulus of the hydrogel, determining it to be 110 kPa, which is lower than the modulus of whole nerves (500–70,000 kPa) and therefore it should not cause any irritations to surrounding tissue after implantation. In a follow-up study, Gander and coworkers coated these tubular hydrogels with layers of poly(lactide-*co*-glycolide) (PLGA) and investigated the release kinetics of nerve grow factors upon acidic degradation of the PLGA layers.[182]

An alternative to biopolymers are mixtures of naturally derived and synthetic precursor materials. Aida and colleagues mixed naturally derived clay nanosheets with negatively charged polyacrylates;[183] upon subsequent addition of macromolecular crosslinkers based on end-group functionalized PEG with dendrimers that bear positively charged guanidinium moieties, translucent hydrogels can be obtained. Clay nanosheets form highly entangled associates with each other, which are disrupted upon addition of anionic polyacrylate, due to a site-specific wrapping of the polymer chains to the positively charged edges of the clay nanosheets. The resulting charged sheets mutually reject each other and as a result are homogenously dispersed in the solution. The addition of a positively charged macromolecular binder interconnects the sheets again, thereby forming a hydrogel, as shown in Figure 8A. Interestingly, these hydrogels have a water content of ≥ 95% and contain only 0.4% of organic materials and 2–3% clay, respectively. Rheological probing of hydrogels with different compositions showed exceptionally high mechanical toughness and a dependence on the dendrimer generation as well as the clay concentration used. Materials that are composed of a binder with [G3]-dendrimers and 5 wt% clay exhibited an elastic modulus of ~$2.5 \cdot 10^5$ Pa, which is considerably higher than previously ionically crosslinked hydrogels. Application of high yield stress resulted in a breakdown of the physical crosslinks, which, however, are almost instantly reformed after withdrawal of the stress. Aida and coworkers used this self-healing ability to adhere hydrogel fragments to each other, allowing for the customization and molding of the hydrogels, as demonstrated in Figure 8B. Furthermore, the solvent of hydrogels can be replaced by immersing the gels into organic solvents or even ionic liquids, allowing for the preparation of novel iono- and organogels. Even though the amount of organic components needed for the preparation of the hydrogels is fairly low, the preparation and functionalization of dendrimers is a time-consuming endeavor. To address this issue, Aida and collaborators employed ABA triblock copolyethers bearing guanidinium moieties in their respective endblocks for the preparation of hydrogels. The mechanical properties of such hydrogels are comparable to the hydrogels crosslinked by dendrimer-based binders, but the synthesis of the block copolymers used as crosslinkers is less laborious.[184]

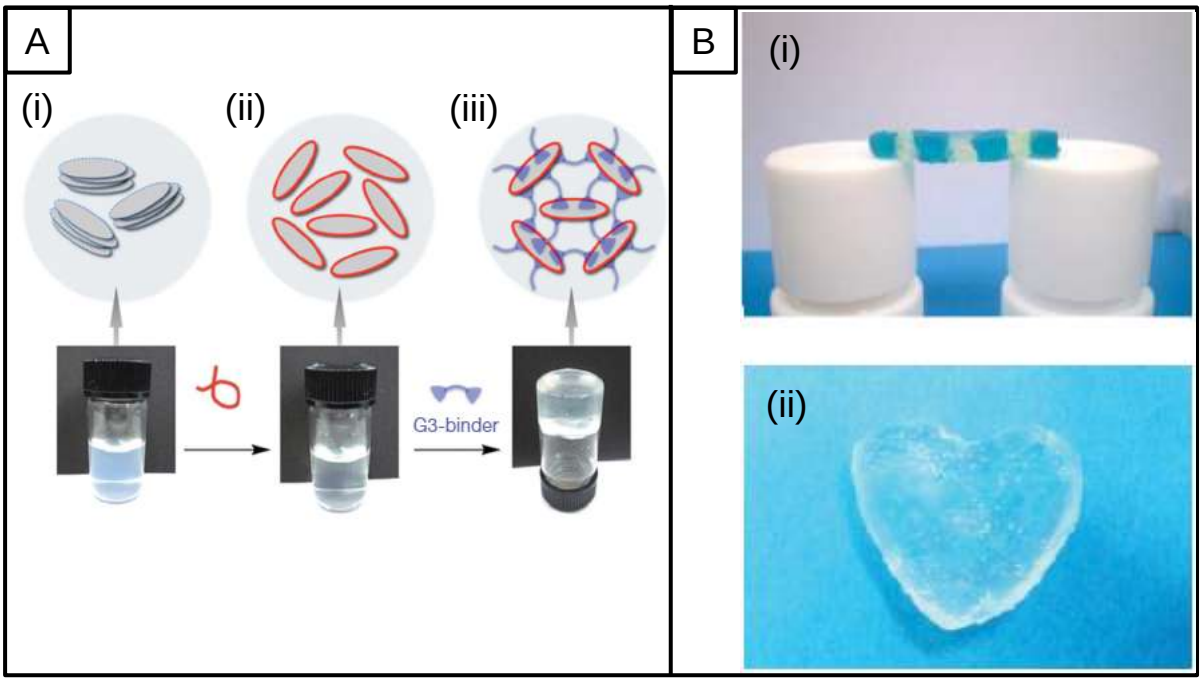


Figure 8. Formation of hydrogels and shape-persistent free-standing macroscopic objects molded from hydrogels. (A) (i) Inhomogeneous dispersion of assembled nanoclay particles in water. (ii) Upon addition of polyacrylate (indicated by the red polymer chain), nanoclay particles are wrapped by the anionic polymer chains and are homogenously dispersed in water. (iii) Addition of dendrimer binder interconnects the clay discs, leading to the formation of macroscopic hydrogels. (B) (i) Free-standing hydrogel prepared from seven separate hydrogel fragments by application of the self-healing properties of the supramolecular hydrogels. (ii) A hydrogel molded into a heart shape. Reprinted with permission from ref. 183. Copyright 2010 Nature Publishing Group.

In a seminal work, Cohen Stuart and colleagues prepared multiresponsive ionically crosslinked hydrogels by mixing a negatively charged triblock copolymer containing a hydrophilic midblock with a positively charged homopolymer.[40] Investigations by DLS and SAXS showed that mixing of these two oppositely charged polymers led to the formation of flowerlike micelles at low concentrations, whereas highly viscous and transparent gels were spontaneously formed after mixing of the polymers at high concentrations as a result of micellular crosslinking. The mechanical properties of these hydrogels can be influenced by charge composition, concentration, ionic strength, pH value, and temperature.

In a similar work, Hawker and coworkers prepared a set of different hydrogels based on oppositely charged ABA triblock copolymers.[166] The block copolymers were composed of a solvophilic PEG midblock and two charged endblocks that contained either positively charged ammonium or guanidinium moieties or negatively charged carboxylate or sulfonate moieties. By this means, Hawker and colleagues investigated the influence of the pK_a on the formation of polymer networks based on the identical polymeric back bone, but containing different functional ionic endgroups. Mixing solutions of copolymers with weaker ionic groups, such as ammonium and carboxylate moieties as well as ammonium and sulfonate moieties, led to the formation of highly viscous solutions, whereas mixing of guanidinium-functionalized triblocks with either carboylate- or sulfonate-functionalized triblocks led to the formation of hydrogels. The latter hydrogel exhibited the highest stability as well as the highest mechanical resilience in these trials and could also be prepared at the lowest concentrations (3–5 wt%). The dynamic responsiveness of the hydrogels was

investigated by exposing them to media of increasing salt concentration, which led to a decrease of the mechanical toughness of the materials as determined by oscillatory shear rheology.

1.2. Hybrid Hydrogels

Supramolecular polymer hydrogels often lack stability due to their transient crosslinking, but excel in stimuli-responsiveness and self-healing properties. In contrast, covalently crosslinked hydrogels often exhibit tough mechanical properties that can be tuned over a wide range, but they become irreversibly damaged when exposed to extensive mechanical stress. The combination of both types of crosslinking negates their individual disadvantages and allows for the development of new materials with unique abilities. Even though the term 'hybrid' is also used for materials that are composed of natural and synthetic polymers or organic and inorganic materials, in this case 'hybrid' is referred to hydrogels that employ both physical interactions and chemical crosslinking independent of their composition.

Hybrid hydrogels can exhibit extraordinary mechanical properties, including high stretchability and toughness. In 2003, Osada and coworkers reported on covalently crosslinked interpenetrating hydrogels with a very high mechanical strength.[185] These so-called 'double networks' exhibited mechanical properties that exceeded those expected of normal interpenetrating networks. This is achieved by the fracturing of polymer chains in the hydrogels as a mechanism to dissipate mechanical energy.[186] As a result, chemically-crosslinked double networks can only undergo a limited number of loading cycles due to fatiguing of the material. Elaborating on this work, Suo and coworkers prepared highly stretchable and tough hydrogels by mixing alginates and PAAm.[187] To achieve this, acrylamide monomers, *N*,*N*'-methylenebisacrylamide, ammonium persulfate, calcium sulfate, and alginate powder were mixed in water and upon irradiation with ultraviolet light hybrid hydrogels were formed. The proposed double network structure consists of calcium-crosslinked alginate domains, covalently crosslinked PAAm domains, and covalently crosslinked alginate-PAAm domains. The hydrogels were then mounted into a tensile machine to investigate the stretchability, resulting in a stretch of 21 times its initial length. Upon inflicting a notch and subsequent stretching, the hydrogels still can be elongated to 17 times their length before critical failures occur, as shown in Figure 9. Then, the stretching experiments were repeated using homopolymer hydrogels prepared from PAAm and alginate, respectively, and the respective elastic moduli were determined. During stretching, the hybrid hydrogels exhibited an elastic modulus close to the combined elastic moduli of the two homopolymer networks. However, at the moment of rupture, the elastic modulus far exceeded those of the homopolymer networks. The authors attributed this finding to the unique network structure of the hydrogels. When the hydrogels are stretched the physical crosslinks of the alginate chains are disrupted, allowing for dissipation of the energy. The chemical crosslinks then bridge these internally occurring cracks and hold the double network structure in place. Upon release of the stress, the network returns to its original shape and the physical crosslinks are reformed again. Critical failure of the hydrogel occurs only after the physical crosslinks are completely broken and the chemical crosslinks start to break.

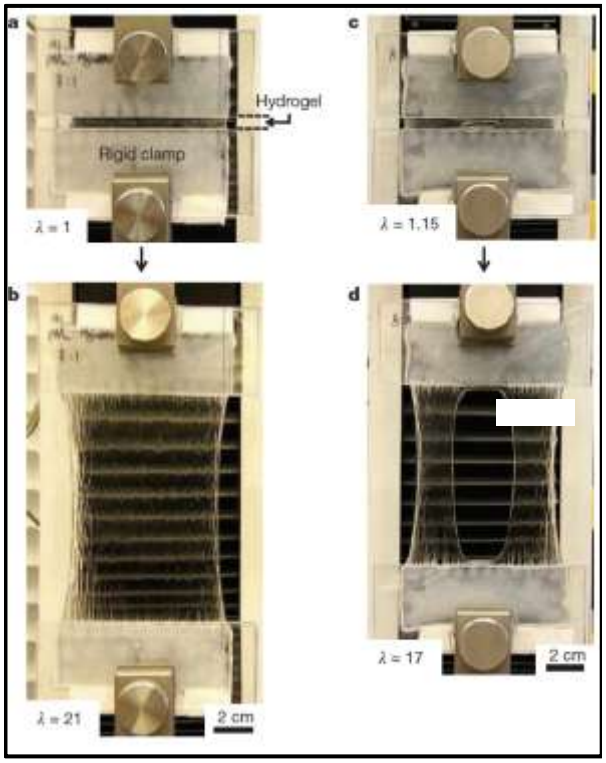


Figure 9. Stretch tests of polymer strips using a tensile machine. (a) A strip of the undeformed hydrogel was glued to two rigid clamps. (b) The gel was stretched to 21 times its initial lengths. The stretch, λ, is defined by the distance between the two clamps when the gel is deformed, divided by the initial distance at which the gel is not deformed. (c) A notch was cut into the gel. A small stretch of 1.15 was made to increase the visibility of the notch. (d) The hydrogel was stretched to 17 times its initial length. Reprinted with permission from ref. 187. Copyright 2012 Nature Publishing Group.

In a similar approach, Panhuis and coworkers developed a hydrogel with a double network structure that can resist high mechanical strains and compressions.[62] Additionally, the gels have the ability to recover after several loading–unloading cycles. For this purpose, hot solutions of gellan gum (GG) and acrylamide were mixed and upon addition of their respective crosslinkers, *N*,*N*′-methylenebisacrylamide and $CaCl_2$, hybrid hydrogels of covalent as well as ionic crosslinks were formed. To investigate if PAAm–GG networks possess a double network structure, hydrogels based on PAAm, GG and PAAm–GG were compressed to failure, showing that the compressive stress for PAAm–GG networks is almost twice as high as the combined stress for the single networks. The self-healing properties of the PAAm–GG hydrogels were investigated by compressive cycles of loading of 5N and subsequent resting after unloading. These experiments showed a strong dependence on the resting time between each cycle with recovery of the mechanical properties ranging from 30% after 2 min to 92% after 7 d. The recovery is possible due to the reformation of the ionic crosslinks, which are temporarily disrupted upon hydrogel compression.

Hybrid hydrogels employing both chemical crosslinking and metal complexation have been investigated to prepare stimuli-responsive materials, which are interesting candidates for drug delivery applications. Additionally, the combination of a stimuli-responsive polymer and complexation of a metal ion that can easily be oxidized and reduced, leads to the possibility of dynamic self-oscillating hydrogels. Yoshida and coworkers prepared a mechanically oscillating hydrogel based on poly(*N*-isopropylacrylamide) (PNIPAM) functionalized with Ru(II)-trisbipyridine moieties in the side-chain.[188] The oscillation is induced by the Belousov–Zhabotinsky (BZ) reaction within the hydrogel. The BZ reaction is the oxidation of an organic substrate (malonic acid) by an

oxidizing agent (bromate ion) in the presence of a strong acid and a metal catalyst. During this reaction, the metal catalyst undergoes spontaneous redox oscillation, which is indicated by a periodic color change of the solution. When Ru(II)-trisbipyridine containing hydrogels are immersed in a solution containing malonic acid, bromate ions, and acid, periodical swelling–deswelling is observed. The swelling–deswelling phenomenon is caused by the volume–phase transition of the PNIPAM backbone. Upon oxidation of Ru(II) to Ru(III), the phase transition temperature of PNIPAM is increased from 32 °C to 36 °C, resulting in swelling of the hydrogel, because of an increase of the hydrophilicity of the PNIPAM backbone. When the ruthenium ions are reduced again, the hydrogel shrinks back to its initial size, as summarized in Figure 10. By this means, the network can generate mechanical energy from the chemical energy of the BZ reaction. This type of material has gained interest for applications as self-beating micropumps, peristaltic microactuators, or pacemakers.[189]

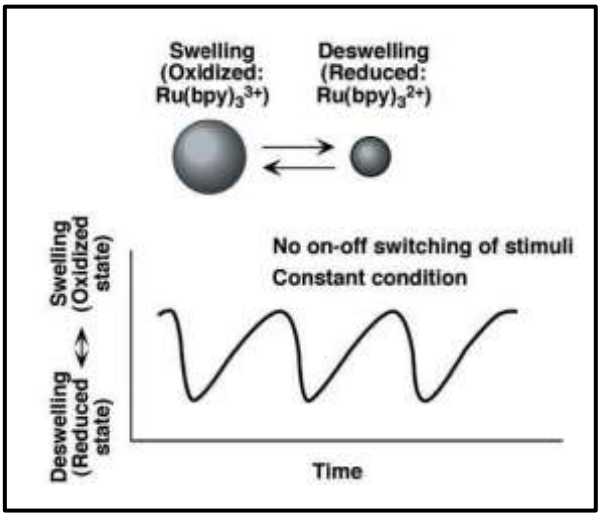


Figure 10. Schematic representation of the Belousov–Zhabotinsky reaction combined with stimuli-responsive hydrogels. An oscillating swelling–deswelling of the hydrogels can be observed upon the cyclic oxidation and reduction of ruthenium ions. Reprinted with permission from ref. 189. Copyright 2010 WILEY-VCH Verlag GmbH & Co KGaA.

Besides extraordinary mechanical properties in combination with self-healing, hybrid hydrogels can exhibit shape-memory abilities. The first polymeric material with shape-memory effect was claimed in a patent by Vernon in 1941, employing a methacrylic ester resin that could resume its original shape upon heating.[190] However, the first hybrid hydrogels with shape-memory were reported on during the 1990's.[191] The mechanism involved in shape-memory relies on the disruption of the reversible supramolecular crosslinks of the hydrogel, thus allowing for molding of the gel into the desired shape and upon reformation of the crosslinks, this shape is fixed until a recurring disruption.[192]

Uragami and coworkers employed this design principle and prepared semi-interpenetrating networks based on poly(acrylamide) (PAAm).[43] To achieve this, antibodies and antigens were functionalized with *N*-succinimidylacrylate and the antibody was copolymerized with acrylamide to yield linear PAAm functionalized with one antibody per polymer chain. Then, the vinyl-functionalized antigen monomer and the antibody-functionalized PAAm were mixed, allowing for the formation of an antibody–antigen complex that is subsequently copolymerized by free radical polymerization using *N*,*N'*-methylenebisacrylamide as well as acrylamide. Comparison of the binding strength of the antibody to native and polymerized antigen revealed a lower binding constant for the polymerized

antigen. This allows for the disruption of the supramolecular crosslinks by addition of native antigen to the hydrogels, resulting in an abrupt swelling of the hydrogels, as depicted in Figure 11. After immersion of these swollen hydrogels in buffer solution, the native antigens diffuse out of the gels and the supramolecular crosslinks are reformed, due to the trapped antibody-functionalized polymer chains in the hydrogels. As a result, the initial hydrogel properties are regained and the gels shrink. This reversible shape-memory can also be employed for controlled permeation of protein drugs through such hydrogels, as demonstrated by Uragami and colleagues. They prepared hydrogel membranes out of this material and determined the permeation of hemoglobin, a model protein drug, during stepwise addition of native antigens, showing that the permeation can be precisely controlled by admission of antigens. This experiment suggests that this material platform might allows for drug delivery in response to specific antigens.

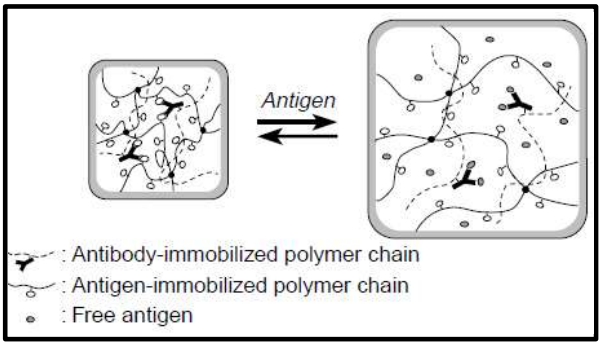


Figure 11. Diagram of the swelling–deswelling mechanism of antigen-responsive hydrogels. Upon addition of native antigens, the supramolecular crosslinks of the hydrogel are disrupted, resulting in swelling of the gel. Reprinted with permission from ref. 43. Copyright 1999 Nature Publishing Group.

Typically, shape-memory materials exhibit two states, a temporal and a permanent one. Liu and coworkers, however, reported on a hybrid hydrogel with shape memory that possesses three different states.[193] For this purpose, hydrogels were prepared based on poly(acrylonitrile-*co*-2-methacryloyloxyethyl phosphorylcholine) (P(AN-*co*-MPC)) by photopolymerization of the respective monomers with *N*,*N*′-methylenebisacrylamide as a crosslinker. Beside covalent crosslinking, the hydrogels are physically crosslinked by dipole–dipole interactions of cyano moieties present in the hydrogels. Upon addition of Zn(II) ions, these interactions can be disrupted in favor of the formation of Zn-cyano metal complexes depending on the Zn(II) concentration added. By this means, the formation of three different supramolecular assemblies is possible. In the native P(AN-*co*-MPC) hydrogel only the dipole–dipole interaction of the cyano groups exist. Upon immersion of the hydrogel in a 30% $ZnCl_2$ solution, additional Zn–dicyano associations are formed, which results in denser polymeric networks, causing shrinkage of the hydrogel. However, when the native hydrogel is immersed into a 50% $ZnCl_2$ solution, all supramolecular crosslinks are disrupted by the formation of zinc–monocyano complexes, which leads to an increase in the swelling degree of the hydrogel, as compiled in Figure 12B. The shape-memory is realized by employing these three assemblies. To achieve this, a hydrogel strip was immersed into an aqueous solution containing 50% $ZnCl_2$ and curled to a spiral-like shape. The temporary shape 1 was fixed by removing the Zn(II) ions, thus reforming the dipole–dipole interactions. After that, the gel was manually deformed to a loose spiral (temporary shape 2), which could be retained as long as no zinc ions were present. To recover the temporary shape 1, the gel was immersed into a solution containing 30% $ZnCl_2$, which led to reformation of the tightly coiled spiral. The permanent shape was regained by adding the hydrogel to

a 50% $ZnCl_2$ solution, which disrupted the supramolecular crosslinks, as summarized in Figure 12A. Moreover, investigations of the mechanical properties of such gels showed that the hydrogels exhibit very high Young's moduli as well as tensile strengths. These properties were retained even after multiple cycles of addition of Zn(II) ions and subsequent immersion in water.

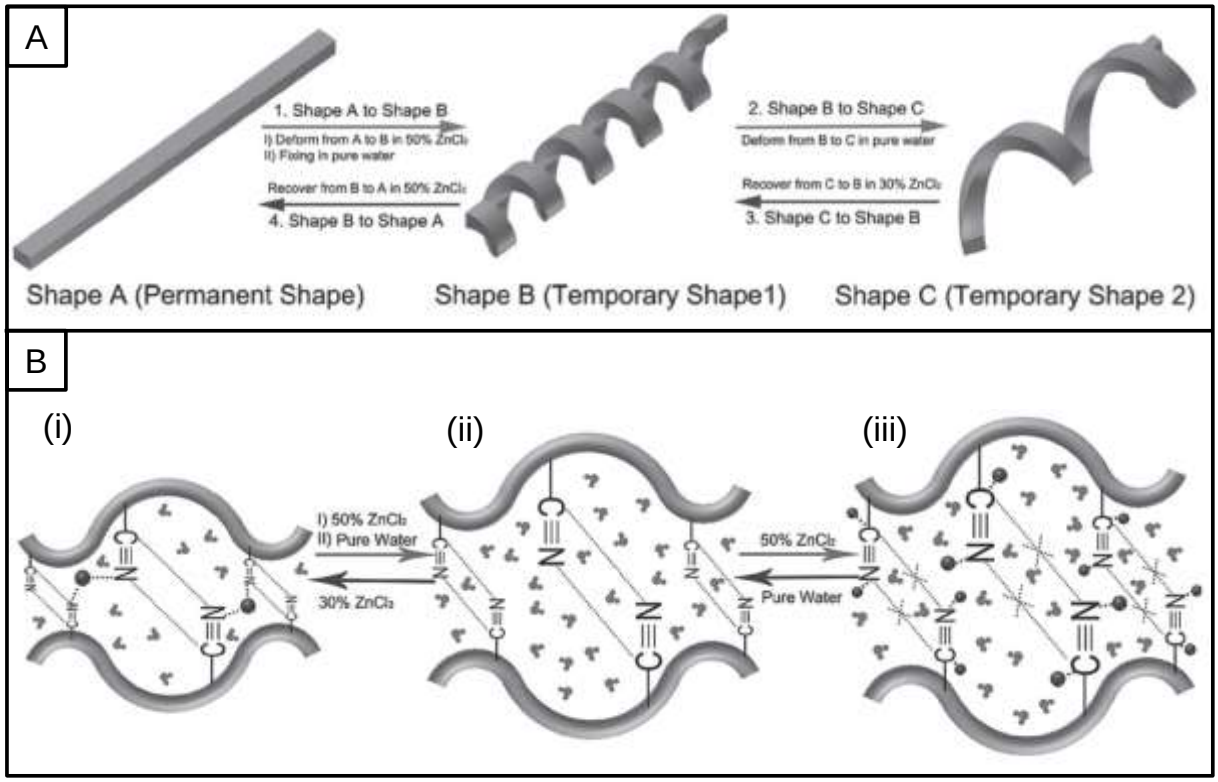


Figure 12. Illustration of the triple shape-memory effect of poly(acrylonitrile-*co*-2-methacryloyloxyethyl phosphorylcholine) hydrogels and the proposed underlying mechanism. (A) 1: A straight hydrogel strip was curled into a tight spiral (Temporary Shape 1) and subsequently fixed in water. 2: The tight spiral was manually deformed into a loose spiral (Temporary Shape 2) in pure water. 3: After immersing in 30% $ZnCl_2$ solution, Temporary Shape 1 was recovered. 4: The permanent shape was recovered by immersion in 50% $ZnCl_2$ solution. (B) (i) In 30% $ZnCl_2$ solution, formation of CN–CN dipoles and of Zn–CN complexes. (ii) In pure water, only dipole–dipole interactions are present. (iii) In 50% $ZnCl_2$, all physical crosslinks are disrupted. Reprinted with permission from ref. 193. Copyright 2011 WILEY-VCH Verlag GmbH & Co KGaA.

Shape-memory like behavior can also be forced by applying an electric field to hydrogels that exhibit high charge densities. In an effort to convert chemical to mechanical energy, Hori and coworkers prepared negatively charged covalently crosslinked hydrogels based on poly(2-acrylamido-2-methylpropanesulfonic acid) (PAMPS) and placed them in an aqueous solution that contained *n*-dodecyl pyridinium chloride, a cationic surfactant.[194] Upon applying an electric field to the aqueous solution, the hydrogel was first stretched and, when the polarity was switched, bend. By altering the polarity in an oscillating manner, a walking motion with a constant velocity of 25 cm min^{-1} could be observed. The mechanism of the stretching is explained as follows: when an electric field is applied, the positively charged surfactant molecules move towards the cathode and form ionic complexes with the negatively charged hydrogel, preferentially on the side of the gel that is facing the anode, causing the hydrogel to bend towards the anode. Upon change of the polarity of the electric field, the ionic complexes are dissolved and the surfactant molecules move toward the anode. The gel is

then straightened by formation of ionic complexes on the opposite side of the hydrogel, as shown in Figure 13. This material can be envisioned as an actuator in artificial muscles.

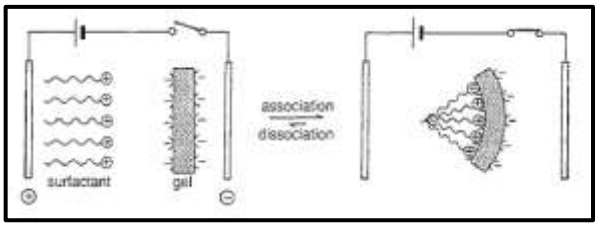


Figure 13. Schematic illustration of the bending mechanism by association of surfactant molecules under electric field. Reprinted with permission from ref. 194. Copyright 1992 Nature Publishing Group.

2. Scientific Goals

The rational design of supramolecular polymer networks necessitates an extensive knowledge about the interplay of their structure, dynamics, and properties. In an effort to achieve this understanding, several material platforms have been developed, which, however, have several major limitations: First, most of these platforms employ only one type of supramolecular interaction, either hydrogen bonding or metal complexation. Second, a common design principle for such polymer networks is the copolymerization of monomers one type of which is modified with a supramolecular crosslinkable motif, allowing for a one-pot synthesis of functional polymers. However, this synthetic procedure leads to a batch-to-batch variation of the resulting polymers in terms of molecular weight and distribution of functional motifs. Third, many existing material platforms are limited to specific solvents due to solubility issues, impairing the potential to investigate the influence of different solvent polarities. This also impedes comparative studies of the kinetics and thermodynamics of network formation and dynamics.

All these limitations obstruct consistent and comprehensive investigation and comparison of different types of supramolecular polymer networks to derive general structure–property relations. The aim of this thesis is to address this issue. To achieve this, a modular polymeric toolkit was developed that employs only *one* backbone polymer, which can be functionalized with either hydrogen bonding or metal complexation motifs without altering the properties of the backbone polymer. Moreover, this material allows for the investigation of the impact of the type and strength of supramolecular chain interconnection in various organic solvents. By this means, network mechanics and dynamics can be studied with unprecedented consistency by employing techniques such as oscillatory shear rheology and fluorescence recovery after photobleaching.

Supramolecular polymeric materials are interesting candidates for life science applications due to their transient crosslinks. For such applications two prerequisites have to be met, mild crosslinking conditions and stability of the crosslinks towards water. In this thesis, a stimuli-responsive polymeric toolkit based on water-soluble and biocompatible polyglycerol was developed that employs supramolecular chain interconnection based on both hydrogen bonding and metal complexation, which are strong enough to resist the competing interaction with water. The transient nature of the crosslinks allowed for the orthogonal decrosslinking of the resulting hydrogels. In combination with the biocompatibility of the polymeric backbone, this polymeric toolkit is a promising candidate for potential use as scaffold for cell encapsulation, manipulation, and release.

In biological systems, various mechanisms control the reaction towards external stimuli, e.g. mechanotransduction for conversion of mechanical stimulation into electrochemical activity. By this means, the differentiation of stem cells can be triggered by abrupt change of the mechanical properties of the surrounding cellular environment. To investigate this, a polymeric toolkit was prepared based on poly(*N*-(2-hydroxypropyl)-methacrylamide) grafted with pendant poly(*N*-isopropylacrylamide) (PNIPAM) side chains and crosslinked by bio-orthogonal azide–alkyne click chemistry of suitably functionalized poly(ethylene glycol) in aqueous media. The resulting hydrogels were able to abruptly change their mechanical properties upon increase of the temperature, because of the volume–phase transition of PNIPAM, thereby combining both chemical and physical crosslinking. The combination of droplet-based microfluidics and bio-orthogonal crosslinking allowed for the preparation of cell-laden stimuli-responsive hybrid microgels, proving them to be a suitable platform for future systematic stem-cell research.

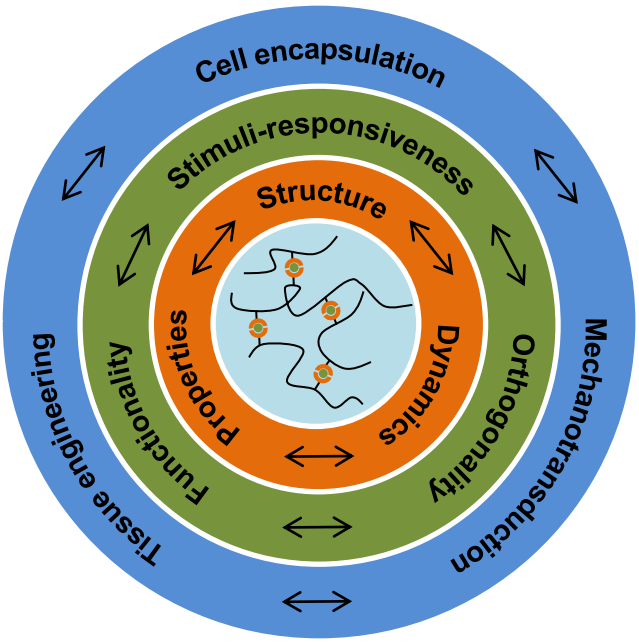
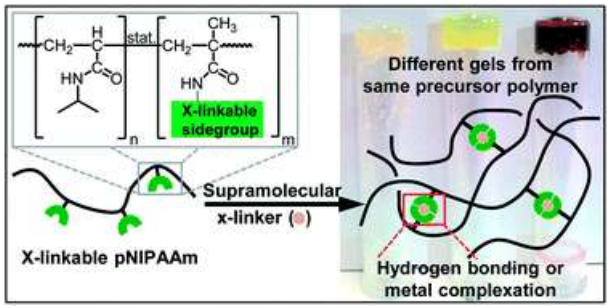


Figure 14. Diagram for the development of functional materials. The inner layer shows the microscopic characteristics than can be tuned to obtain the properties that are described in the middle layer. By considering these two layers, hydrogels can be tailor-made to meet the prerequisites of the applications in the outer layer.

3. Publications

3.1. A Modular Construction Kit for Supramolecular Polymer Gels



T.Rossow, S. Hackelbusch, P. van Assenbergh, and S. Seiffert, *Polym. Chem.* **2013**, *4*, 2515–2527.

http://dx.doi.org/10.1039/C3PY00104K

Author contributions

T. Rossow: Project development, polymer synthesis, synthesis of supramolecular crosslinkable motifs, polymer functionalization, gelation studies, analyses of data from isothermal titration calorimetry (ITC), preparation of the manuscript.

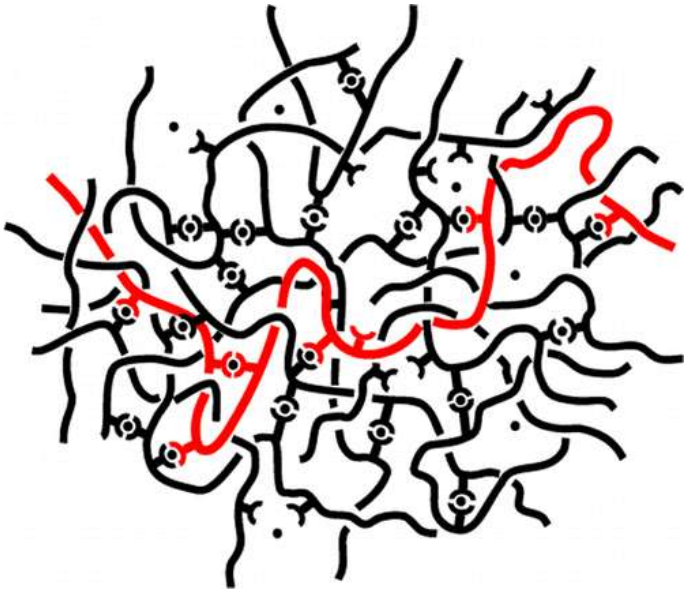
S. Hackelbusch: Polymer synthesis, rheology and ITC measurements, preparation of the manuscript.

P. van Assenbergh: Rheology measurements.

S. Seiffert: Conception and supervision of the work, rheology data analysis, preparation of the manuscript.

In this manuscript, a modular construction kit for the preparation of supramolecular polymer networks was developed. To achieve this, linear copolymers based on electrophilic *N*-(methacryloyloxy)succinimide (MASI) and *N*-isopropylacrylamide (NIPAM) were prepared by controlled radical polymerization. The MASI units were then substituted by addition of amine-functionalized supramolecular crosslinkable motifs. By this means, a set of PNIPAM polymer based on the same backbone polymer with different crosslinkable sidegroups was prepared. Polymer chain association was achieved via hydrogen bonding or metal complexation upon addition of low molecular crosslinkers that are complementary to the functional side groups on the polymer chains. Hydrogen bonding was based on the interactions of diaminotriazine and maleimide, cyanuric acid and Hamilton wedges, and diaminotrizine and cyanuric acid. Metal complexation, on the other hand, was based on terpyridine and various metal salts. As a result, supramolecular polymer networks were formed with varying mechanical properties, ranging from low viscous polymer solutions to highly elastic organogels, depending on the binding strength of the supramolecular interaction.

3.2. Chain Dynamics in Supramolecular Polymer Networks



S. Hackelbusch, T. Rossow, P. Van Assenbergh, and S. Seiffert, *Macromolecules* **2013**, *46*, 6273–6286.

http://dx.doi.org/10.1021/ma4003648

Author contributions

S. Hackelbusch: Polymer synthesis and functionalization, rheology measurements, fluorescence recovery after photobleaching experimentation.

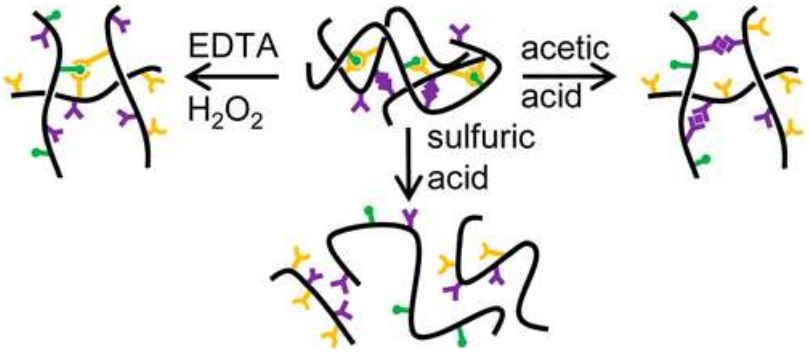
T. Rossow: Synthesis of the supramolecular crosslinkable motifs.

P. Van Assenbergh: Rheological measurements.

S. Seiffert: Conception and supervision of the work, fluorescence recovery after photobleaching experimentation and data analysis, rheology data analysis, physical-chemical picturing, preparation of the manuscript.

In this manuscript, a modular polymeric toolkit was used to prepare supramolecular networks that exhibit varying mechanical properties, ranging from low viscous polymer solutions to highly elastic organogels, depending on the binding strength of their respective transient crosslinking. This design principle allowed for the investigation of the impact of the strength of the chain crosslinking on the network dynamics and mechanics. For this purpose, a fluorescently tagged polymer chain based on the same polymeric material as the polymer networks themselves was used to determine the diffuse mobility within these transient networks. Moreover, the mechanical properties of such networks were studied by oscillatory shear rheology. The combination of both methods showed a concentration dependence of chain diffusivity on the binding strength of the associations. This finding is in agreement to the 'sticky reptation' model by Rubinstein and Semenov for systems with a binding strength higher than 10^9 M^{-2}. Below this threshold, semidilute-solution-type chain dynamics are observed.

3.3. Multiresponsive Polymer Hydrogels by Orthogonal Supramolecular Chain Cross-Linking



S. Hackelbusch,* T. Rossow, H. Becker, and S. Seiffert, *Macromolecules* **2014**, *47*, 4028–4036.
[*equal contribution]

http://dx.doi.org/10.1021/ma5008573

Author contributions

S. Hackelbusch: Synthesis of linear polyglycerol and of cyclooctyne derivatives, polymer functionalization, rheology measurements, preparation of the manuscript.

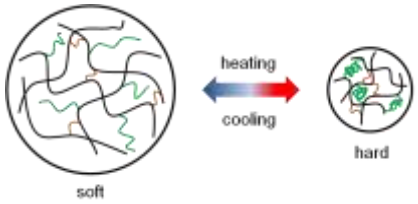
T. Rossow: Project conception and development, synthesis of supramolecular crosslinkable motifs and of linear polyglycerol, polymer functionalization, rheology measurements, de-crosslinking studies, preparation of the manuscript.

H. Becker: Preliminary work, synthesis of supramolecular crosslinkable motifs and of linear polyglycerol.

S. Seiffert: Supervision, correction of the manuscript.

In this manuscript, a polymeric toolkit was developed for the preparation of hydrogels crosslinked by either hydrogen bonding, metal complexation, or both. For this purpose, biocompatible linear polyglycerol was functionalized with cyanurate and diaminotriazine moieties that can form a multipoint hydrogen-bonding array, along with terpyridine moieties that can complex Fe(II)-ions. Gelation occurred by mixing of aqueous polymer solutions and subsequent addition of Fe(II) ions. The resulting hydrogel were stable in water for several weeks. The orthogonality of this approach allowed for the selective decrosslinking the hydrogels by addition of acetic acid in case of the hydrogen bonding or addition of a metal chelator for disrupting of the metal complexes. Complete disruption of the hydrogels was achieved by addition of the sulfuric acid. The orthogonality of the crosslinking in combination with the biocompatibility of the material renders this toolkit a promising alternative for the encapsulation and subsequent controlled release of cells.

3.4. Hybrid Microgels with Thermo-Tunable Elasticity for Controllable Cell Confinement



S. Hackelbusch, T. Rossow, D. Steinhilber, D.A. Weitz, and S. Seiffert, *Adv. Healthcare Mater.* **2015**, *4*, 1841–1848.

http://dx.doi.org/10.1002/adhm.201500359

Author contributions

S. Hackelbusch: Conception of the work, project development, synthesis, gelation studies, rheology measurement, cell encapsulation, preparation of the manuscript.

T. Rossow: Synthesis of PEG-diamine, microfluidic templating and cell encapsulation, confocal laser scanning microscopy, preparation of the manuscript.

D. Steinhilber: Microfluidic templating, stem cell culturing.

D.A. Weitz: Correction of the manuscript.

S. Seiffert: Conception and supervision of the work, static light scattering, correction of the manuscript.

In this manuscript, we developed a hybrid toolkit for the preparation of stimuli-responsive hydrogels that are chemically crosslinked, but also allow for additional physical crosslinking. For this purpose, *N*-(2-hydroxypropyl)-methylacrylamide (HPMA) and hydroxyethyl methacrylate (HEMA) were copolymerized. Then, the terminal hydroxyl groups of the HEMA units were converted to azides in a two-step protocol. The azide moieties allowed for the functionalization with PNIPAM and the chemical crosslinking with cyclooctyne-functionalized PEG, both by bio-orthogonal strain-promoted azide–alkyne click chemistry. By combination of this crosslinking chemistry and droplet-based microfluidic, cell-laden microgels were obtained that exhibited cell viabilities higher than 90%. Upon increase of the temperature from 32 °C to 37 °C, the PNIPAM chains underwent a volume–phase transition, thus leading to a contraction of the microgels and an increase of the elastic modulus. The hydrogels can be customized further by tuning the initial elastic modulus at low temperatures or addition of protein sequences as anchoring points for cells. As a basis for future applications of this hybrid toolkit for the preparation of extracellular matrixes that are suitable to induce and study stem cell differentiation, mesenchymal stem cells were successfully encapsulated and showed cell viabilities higher than 80% after encapsulation.

4. Summary and Conclusions

In this thesis, a modular polymeric toolkit was developed, which allows for the preparation and investigation of supramolecular polymer gels in various solvents to derive general structure-property relationships. To achieve this, *N*-isopropylacrylamide (NIPAM) monomers were copolymerized with electrophilic *N*-(methacryloyloxy)succinimide (MASI) monomers by controlled radical polymerization, yielding a PNIPAM copolymer with a narrow molecular weight distribution. The MASI units were replaced by nucleophilic substitution with amine-functionalized supramolecular bonding motifs, resulting in a set of supramolecularly crosslinkable polymers based on the same backbone material. Polymer networks were obtained by crosslinking via either hydrogen bonding or metal complexation upon addition of low molecular crosslinkers that are complementary to the moieties on the functional polymer chains. Combined quantitative measurements of oscillatory shear rheology and isothermal titration calorimetry showed a dependence of the mechanical properties of such polymer networks from the strength of the supramolecular crosslinks. As a result, supramolecular networks of varying strength, from low viscous solutions to highly elastic gels, could be obtained.

Besides supramolecular crosslinkable motifs, the polymer backbone has been functionalized with amine-functionalized fluorophores, offering the possibility to probe polymeric networks by fluorescence-based imaging or tracking techniques. By this means, polymer networks were investigated with respect to the micrometer-scale mobility of linear polymer chains diffusing through polymer networks of varying strength. The results were compared to the predictions of the 'sticky reptation' model by Rubinstein and Semenov, indicating a certain threshold strength of supramolecular association, where the concentration dependence of the tracer-chains diffusivity is in agreement with this model.

During these trials, we found that multipoint hydrogen-bonded arrays based on cyanurate and diaminotriazine have extraordinary binding strengths, even in polar solvents such as water. As a result, we functionalized biocompatible and water-soluble polyglycerol with either cyanurate or diaminotriazine and with terpyridine. This design principle allows for the orthogonal crosslinking of these polymer chains under mild gelation conditions by mixing aqueous solutions of these polymers and by subsequently adding Fe(II) ions. The resulting hydrogels were stable in water for several weeks. The orthogonality of this approach allows for the decrosslinking of such hydrogels by selective stimulation. For this purpose, a metal chelator that selectively disrupted the Fe(II)–terpyridine complexes was added and addition of acetic acid reversed the hydrogel bonding without interfering with metal complexation. The disruption of both types of crosslinking at the same time was achieved by sulfuric acid. The orthogonality of crosslinking in addition to the biocompatibility of the polyglycerol backbone render this material promising as a platform for temporary cell encapsulation and controlled cell release.

In addition to cell encapsulation, stimuli-responsive polymeric materials can also be useful to manipulate confined cells when a certain trigger is applied. To achieve this, we designed a hybrid polymeric toolkit that combines both chemical and physical crosslinking. For this purpose, thermo-responsive PNIPAM was grafted on a poly(*N*-(2-hydroxypropyl)-methylacrylamide) (PHPMA) backbone that was covalently crosslinked by bioorthogonal strain-promoted azide–alkyne cycloaddition with cyclooctyne-functionalized PEG. This crosslinking chemistry in combination with droplet-based microfluidic templating allowed for the preparation of cell-laden microgels with cell viabilities higher than 90%. Upon change of the temperature from 32 °C to 37 °C, the dangling PNIPAM chains underwent a volume–phase transition, causing contraction of the microgels and

increasing the elastic modulus. The design principle of these hydrogels allows for tuning the low-temperature elasticity as well as the further functionalization of the PHPMA backbone, e.g., with peptide sequences as anchoring points for cells. The combination of this customizable design with the reversible switchability of the mechanical properties renders this material platform an interesting candidate for induction and study of stem-cell differentiation.

5. Zusammenfassung und Fazit

In dieser Arbeit wurde ein modularer Polymerbaukasten entwickelt, der es erlaubt, supramolekular vernetzte Polymergele in verschiedenen Lösungsmitteln herzustellen und zu untersuchen. Mit Hilfe dieser Untersuchungen konnten dann die grundlegenden Beziehungen zwischen der Struktur der Gele und ihren jeweiligen Eigenschaften aufgeklärt werden. Um das zu erreichen, wurde zunächst über kontrollierte radikalische Polymerisation ein Copolymer bestehend aus *N*-Isopropylacrylamid (NIPAM) und elektrophilen *N*-(Methacryloyloxy)succinimid (MASI) Monomeren hergestellt. Die MASI Einheiten konnten dann mittels nukleophiler Substitution durch Amin-funktionalisierte supramolekular vernetzbare Motive ersetzt werden. Dadurch konnte ein Satz von Polymeren hergestellt werden, die auf demselben polymeren Ausgangsmaterial basieren, aber unterschiedliche supramolekular vernetzbare Seitengruppen besitzen. Die funktionellen Polymerketten konnten bei Zugabe von niedrigmolekularen komplementären Vernetzern entweder über Wasserstoffbrückenbindungen oder Metallkomplexierung verknüpft werden, was zu einer Bildung von Polymernetzwerken führte. Die Kombination aus quantitativen Messungen mittels oszillatorischer Scherrheologie und isothermaler Titrationskalorimetrie zeigte eine Abhängigkeit der mechanischen Eigenschaften der Polymernetzwerke von der Stärke der supramolekularen Verknüpfung. Auf diese Weise konnten supramolekulare Netzwerke mit unterschiedlichen mechanischen Eigenschaften, von niedrigviskosen Lösungen bis hin zu hochelastischen Gelen, hergestellt werden.

Neben supramolekular vernetzbaren Gruppen konnte das Polymerrückgrat auch mit Amin-funktionalisierten Fluorophoren funktionalisiert werden, was die Möglichkeit bietet, Polymernetzwerke mithilfe von fluoreszenzbasierten Bildgebungsverfahren zu untersuchen. Auf diesem Weg wurde die Diffusion von linearen Polymerketten durch Polymernetzwerke mit unterschiedlichen mechanischen Eigenschaften untersucht. Die Ergebnisse dieser Studie wurden dann mit den theoretischen Vorhersagen des "sticky reptation" Modells von Rubinstein und Semenov verglichen und es konnte gezeigt werden, dass ab einer bestimmten Bindungsstärke der supramolekularen Motive die Konzentrationsabhängigkeit der Diffusivität der Polymerketten mit dem Modell übereinstimmt.

Während dieser Studie fanden wir heraus, dass die komplexe Wasserstoffbrückenbindung basierend auf Cyanursäure und Diaminotriazin eine sehr hohe Bindungskonstante hat, sogar in polaren Lösungsmitteln wie Wasser. Diesen Umstand macht wir uns zunutze, in dem wir biokompatibles und wasserlösliches Polyglycerin sowohl mit Cyanursäure als auch Diaminotriazin und Terpyridin funktionalisierten. Dieses Designprinzip ermöglichte es uns, die supramolekulare Vernetzung der einzelnen Polymerketten unabhängig voneinander unter milden Bedingungen durchzuführen. Hierzu wurden wässrige Polymerlösungen miteinander gemischt und anschließend wurden Fe(II)-Ionen dazugegeben. Die daraus resultierenden Hydrogele waren für mehrere Wochen stabil in Wasser. Die Orthogonalität dieses Ansatzes gestattet es, die supramolekulare Vernetzung selektiv aufzulösen. Um das zu verdeutlichen, haben wir Metall-Chelatoren, welche die Fe(II)-Terpyridinkomplexe auflösen, hinzugegeben und es konnte gezeigt werden, dass die Wasserstoffbrückenbindungen davon nicht beeinflusst wurden. Die Wasserstoffbrückenbindungen hingegen wurden mithilfe von Essigsäure aufgelöst. Die gleichzeitige Auflösung beider supramolekularer Vernetzungen konnte mit Schwefelsäure gezeigt werden. Die Kombination aus der Orthogonalität des Ansatzes und der Biokompatibilität des Polymerrückgrats machen dieses Material

interessant für die Verwendung als Matrix zur Zellverkapselung und der anschließenden kontrollierten Zellfreisetzung.

Zusätzlich zur Zellverkapselung können polymere Materialien, die auf verschiedene Stimulationen reagieren, auch verwendet werden, um in die Matrix eingeschlossene Zellen zu manipulieren. Wir haben diesen Ansatz verfolgt und ein hybrides Baukastensystem entwickelt, welches sowohl chemische als auch physikalische Vernetzung kombiniert. Hierzu wurden thermoresponsive PNIPAM-Ketten an ein Poly(*N*-(2-Hydroxypropyl)-Methacrylamid) (PHPMA) Rückgrat gekoppelt und dieses mithilfe von bioorthogonaler ringspannungsvermittelter Azid–Alkin Zykloaddition mit Cyclooctin funktionalisiertem PEG chemisch verknüpft. Diese Vernetzungschemie in Kombination mit tröpfchenbasierter Mikrofluidik erlaubte es uns, erfolgreich Zellen in Mikrogelen zu verkapseln, welche anschließend eine Überlebensrate von über 90% zeigten. Wenn die Umgebungstemperatur der Mikrogele von 32 °C auf 37 °C erhöht wurde, konnte ein Volumen–Phasenübergang der PNIPAM Seitenketten beobachtet werden, welcher eine Kontraktion der Mikrogele und eine damit verbundene Erhöhung des elastischen Moduls zu Folge hatte. Das Baukastenprinzip erlaubt sowohl die Anpassung des elastischen Moduls bei niedrigen Temperaturen als auch die weitere Funktionalisierung mit zum Beispiel Peptidsequenzen, die als Ankerpunkte für Zellen fungieren können. Die Kombination aus individueller Anpassung des Systems an verschiedene Vorrausetzungen und die reversible Umschaltbarkeit der mechanischen Eigenschaften machen dieses System zu einem interessanten Material für die Untersuchung der Differenzierung von Stammzellen.

6. References

[1] Hackelbusch, S., Seiffert, S., in *In-Situ Gelling Polymers*, Springer Science & Business Media, **2015**, pp. 151-185.

[2] Peppas, N. A., Bures, P., Leobandung, W., Ichikawa, H., *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27-46.

[3] Hennink, W., Van Nostrum, C., *Adv. Drug Delivery. Rev.* **2012**, *64*, 223-236.

[4] Hoare, T. R., Kohane, D. S., *Polymer* **2008**, *49*, 1993-2007.

[5] Tabata, Y., Ikada, Y., *Pharm. Res.* **1989**, *6*, 422-427.

[6] Mather, B. D., Viswanathan, K., Miller, K. M., Long, T. E., *Prog. Polym. Sci.* **2006**, *31*, 487-531.

[7] Nandivada, H., Jiang, X., Lahann, J., *Adv. Mater.* **2007**, *19*, 2197-2208.

[8] Wichterle, O., Lim, D., *Nature* **1960**, *185*, 117-118.

[9] Langer, R., Peppas, N., *Biomaterials* **1981**, *2*, 201-214.

[10] Peppas, N. A., Hilt, J. Z., Khademhosseini, A., Langer, R., *Adv. Mater.* **2006**, *18*, 1345-1360.

[11] Oelker, A. M., Morey, S. M., Griffith, L. G., Hammond, P. T., *Soft Matter* **2012**, *8*, 10887-10895.

[12] White, S. R., Sottos, N. R., Geubelle, P. H., Moore, J. S., Kessler, M. R., Sriram, S. R., Brown, E. N., Viswanathan, S., *Nature* **2001**, *409*, 794-797.

[13] Maldonado-Codina, C., Efron, N., *Ophthalmic Physiol. Opt.* **2004**, *24*, 551-561.

[14] Sun, J.-Y., Zhao, X., Illeperuma, W. R. K., Chaudhuri, O., Oh, K. H., Mooney, D. J., Vlassak, J. J., Suo, Z., *Nature* **2012**, *489*, 133-136.

[15] Yiu, C., Tay, F., King, N., Pashley, D., Sidhu, S., Neo, J., Toledano, M., Wong, S., *J. Dent. Res.* **2004**, *83*, 283-289.

[16] Haraguchi, K., Takehisa, T., Fan, S., *Macromolecules* **2002**, *35*, 10162-10171.

[17] Goycoolea, F. M., Heras, A., Aranaz, I., Galed, G., Fernández-Valle, M. E., Argüelles-Monal, W., *Macromol. Biosci.* **2003**, *3*, 612-619.

[18] Appel, E. A., del Barrio, J., Loh, X. J., Scherman, O. A., *Chem. Soc. Rev.* **2012**, *41*, 6195-6214.

[19] Krische, M., Lehn, J.-M., in *Molecular Self-Assembly Organic Versus Inorganic Approaches,* Springer Berlin Heidelberg, **2000**, pp. 3-29.

[20] Steed, J. W., Atwood, J. L., in *Supramol. Chem.*, John Wiley & Sons, Ltd, **2009**, pp. 49-104.

[21] Piepenbrock, M.-O. M., Lloyd, G. O., Clarke, N., Steed, J. W., *Chem. Rev.* **2009**, *110*, 1960-2004.

[22] Schubert, U. S., Eschbaumer, C., *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 2892-2926.

[23] Berger, J., Reist, M., Mayer, J., Felt, O., Peppas, N., Gurny, R., *Eur. J. Pharm. Biopharm.* **2004**, *57*, 19-34.

[24] Rastello De Boisseson, M., Leonard, M., Hubert, P., Marchal, P., Stequert, A., Castel, C., Favre, E., Dellacherie, E., *J. Colloid Interface Sci.* **2004**, *273*, 131-139.

[25] Abdurrahmanoglu, S., Can, V., Okay, O., *Polymer* **2009**, *50*, 5449-5455.

[26] Abdurrahmanoglu, S., Cilingir, M., Okay, O., *Polymer* **2011**, *52*, 694-699.

[27] Shibayama, M., Tanaka, T., in *Responsive Gels: Volume Transitions I, Vol. 109*, Springer Berlin Heidelberg, **1993**, pp. 1-62.

[28] Rossow, T., Seiffert, S., in *Supramolecular Polymer Networks and Gels*, Springer Science & Business Media, **2015**, pp. 1-46.

[29] Varshey, D. B., Sander, J. R. G., Friščić, T., MacGillivray, L. R., in *Supramol. Chem.*, John Wiley & Sons, Ltd, **2012**.

[30] Brochu, A. B. W., Craig, S. L., Reichert, W. M., *J. Biomed. Mater. Res. A* **2011**, *96A*, 492-506.

[31] van Gemert, G. M. L., Peeters, J. W., Söntjens, S. H. M., Janssen, H. M., Bosman, A. W., *Macromol. Chem. Phys.* **2012**, *213*, 234-242.

[32] Bode, S., Zedler, L., Schacher, F. H., Dietzek, B., Schmitt, M., Popp, J., Hager, M. D., Schubert, U. S., *Adv. Mater.* **2013**, *25*, 1634-1638.

[33] Harrington, M. J., Masic, A., Holten-Andersen, N., Waite, J. H., Fratzl, P., *Science* **2010**, *328*, 216-220.

[34] Lee, H., Dellatore, S. M., Miller, W. M., Messersmith, P. B., *Science* **2007**, *318*, 426-430.
[35] Hager, M. D., Greil, P., Leyens, C., van der Zwaag, S., Schubert, U. S., *Adv. Mater.* **2010**, *22*, 5424-5430.
[36] Capadona, J. R., Shanmuganathan, K., Tyler, D. J., Rowan, S. J., Weder, C., *Science* **2008**, *319*, 1370-1374.
[37] Kabiri, K., Omidian, H., Zohuriaan-Mehr, M. J., Doroudiani, S., *Polym. Compos.* **2011**, *32*, 277-289.
[38] Satarkar, N. S., Zach Hilt, J., *Acta Biomater.* **2008**, *4*, 11-16.
[39] Ye, K. Y., Sullivan, K. E., Black, L. D., *J. Vis. Exp.* **2011**, *55*, e3251.
[40] Lemmers, M., Sprakel, J., Voets, I. K., van der Gucht, J., Cohen Stuart, M. A., *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 708-711.
[41] Tokarev, I., Minko, S., *Soft Matter* **2009**, *5*, 511-524.
[42] Grassi, G., Farra, R., Caliceti, P., Guarnieri, G., Salmaso, S., Carenza, M., Grassi, M., *American Journal of Drug Delivery* **2005**, *3*, 239-251.
[43] Miyata, T., Asami, N., Uragami, T., *Nature* **1999**, *399*, 766-769.
[44] Kuckling, D., *Colloid Polym. Sci.* **2009**, *287*, 881-891.
[45] Hofmeier, H., Schubert, U. S., *Chem. Soc. Rev.* **2004**, *33*, 373-399.
[46] Alonso, M. J., Sánchez, A., *J. Pharm. Pharmacol.* **2003**, *55*, 1451-1463.
[47] LaVan, D. A., McGuire, T., Langer, R., *Nat. Biotechnol.* **2003**, *21*, 1184-1191.
[48] Zheng, B., Wang, F., Dong, S., Huang, F., *Chem. Soc. Rev.* **2012**, *41*, 1621-1636.
[49] Augst, A. D., Kong, H. J., Mooney, D. J., *Macromol. Biosci.* **2006**, *6*, 623-633.
[50] Hashimoto, T., Suzuki, Y., Tanihara, M., Kakimaru, Y., Suzuki, K., *Biomaterials* **2004**, *25*, 1407-1414.
[51] Censi, R., Di Martino, P., Vermonden, T., Hennink, W. E., *J. Control. Release* **2012**, *161*, 680-692.
[52] Drury, J. L., Mooney, D. J., *Biomaterials* **2003**, *24*, 4337-4351.
[53] Langer, R., *Acc. Chem. Res.* **1999**, *33*, 94-101.
[54] Lee, K. Y., Mooney, D. J., *Chem. Rev.* **2001**, *101*, 1869-1880.
[55] Chen, S., Cao, Q., Jing, B., Cai, Y., Liu, P., Hu, J., *J. Appl. Polym. Sci.* **2006**, *102*, 5224-5231.
[56] Chen, S., Hu, J., Zhuo, H., Yuen, C., Chan, L., *Polymer* **2010**, *51*, 240-248.
[57] Huang, W. M., Ding, Z., Wang, C. C., Wei, J., Zhao, Y., Purnawali, H., *Mater. Today* **2010**, *13*, 54-61.
[58] Kersey, F. R., Loveless, D. M., Craig, S. L., *J. Royal Soc. Interface* **2007**, *4*, 373-380.
[59] Kopeček, J., *Biomaterials* **2007**, *28*, 5185-5192.
[60] Wang, C., Kopecek, J., Stewart, R. J., *Biomacromolecules* **2001**, *2*, 912-920.
[61] Joung, Y. K., Ooya, T., Yamaguchi, M., Yui, N., *Adv. Mater.* **2007**, *19*, 396-400.
[62] Bakarich, S. E., Pidcock, G. C., Balding, P., Stevens, L., Calvert, P., *Soft Matter* **2012**, *8*, 9985-9988.
[63] Ashley, M., McCullagh, A., Sweet, C., *Dent. Update* **2005**, *32*, 169.
[64] Fu, Y., Kao, W. J., *J. Biomed. Mater. Res. A* **2011**, *98A*, 201-211.
[65] Ishida, K., Kuroda, R., Miwa, M., Tabata, Y., Hokugo, A., Kawamoto, T., Sasaki, K., Doita, M., Kurosaka, M., *Tissue Eng.* **2007**, *13*, 1103-1112.
[66] Sakai, S., Ito, S., Inagaki, H., Hirose, K., Matsuyama, T., Taya, M., Kawakami, K., *Biomicrofluidics* **2011**, *5*, 013402.
[67] Tabata, Y., Ikada, Y., *Adv. Drug Delivery. Rev.* **1998**, *31*, 287-301.
[68] Bhattarai, N., Gunn, J., Zhang, M., *Adv. Drug Delivery. Rev.* **2010**, *62*, 83-99.
[69] Dumitriu, S., Chornet, E., *Biotechnol. Prog.* **1997**, *13*, 539-545.
[70] Dornish, M., Kaplan, D., Skaugrud, Ø., *Ann. N. Y. Acad. Sci.* **2001**, *944*, 388-397.
[71] Lewen, G., Lindsay, S., Tao, N., Weidlich, T., Graham, R., Rupprecht, A., *Biopolymers* **1986**, *25*, 765-770.
[72] Patel, P., Stripp, A., Fry, J., *Int. J. Food Sci. Technol.* **1988**, *23*, 57-63.
[73] Brandl, F., Sommer, F., Goepferich, A., *Biomaterials* **2007**, *28*, 134-146.

[74] Fiore, G. L., Klinkenberg, J. L., Pfister, A., Fraser, C. L., *Biomacromolecules* **2008**, *10*, 128-133.
[75] Rossow, T., Bayer, S., Albrecht, R., Tzschucke, C. C., Seiffert, S., *Macromol. Rapid Commun.* **2013**.
[76] Kopeček, J., Yang, J., *Polym. Int.* **2007**, *56*, 1078-1098.
[77] Calderón, M., Quadir, M. A., Sharma, S. K., Haag, R., *Adv. Mater.* **2010**, *22*, 190-218.
[78] Dernedde, J., Rausch, A., Weinhart, M., Enders, S., Tauber, R., Licha, K., Schirner, M., Zügel, U., von Bonin, A., Haag, R., *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 19679-19684.
[79] Sisson, A. L., Steinhilber, D., Rossow, T., Welker, P., Licha, K., Haag, R., *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 7540-7545.
[80] Chen, G., Jiang, M., *Chem. Soc. Rev.* **2011**, *40*, 2254-2266.
[81] Guo, M., Jiang, M., Pispas, S., Yu, W., Zhou, C., *Macromolecules* **2008**, *41*, 9744-9749.
[82] Lehn, J.-M., *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89-112.
[83] Aida, T., Meijer, E. W., Stupp, S. I., *Science* **2012**, *335*, 813-817.
[84] Brunsveld, L., Folmer, B. J. B., Meijer, E. W., Sijbesma, R. P., *Chem. Rev.* **2001**, *101*, 4071-4098.
[85] De Greef, T. F. A., Smulders, M. M. J., Wolffs, M., Schenning, A. P. H. J., Sijbesma, R. P., Meijer, E. W., *Chem. Rev.* **2009**, *109*, 5687-5754.
[86] Fox, J. D., Rowan, S. J., *Macromolecules* **2009**, *42*, 6823-6835.
[87] Zeng, F., Zimmerman, S. C., *Chem. Rev.* **1997**, *97*, 1681-1712.
[88] Moore, J. S., *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 108-116.
[89] Noro, A., Hayashi, M., Matsushita, Y., *Soft Matter* **2012**, *8*, 6416-6429.
[90] Seiffert, S., Sprakel, J., *Chem. Soc. Rev.* **2012**, *41*, 909-930.
[91] Beijer, F. H., Kooijman, H., Spek, A. L., Sijbesma, R. P., Meijer, E. W., *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 75-78.
[92] Cooke, G., Rotello, V. M., *Chem. Soc. Rev.* **2002**, *31*, 275-286.
[93] Lehn, J.-M., *Polym. Int.* **2002**, *51*, 825-839.
[94] Nair, K. P., Breedveld, V., Weck, M., *Macromolecules* **2011**, *44*, 3346-3357.
[95] Prins, L. J., Reinhoudt, D. N., Timmerman, P., *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2382-2426.
[96] Jorgensen, W. L., Pranata, J., *J. Am. Chem. Soc.* **1990**, *112*, 2008-2010.
[97] Pranata, J., Wierschke, S. G., Jorgensen, W. L., *J. Am. Chem. Soc.* **1991**, *113*, 2810-2819.
[98] Appel, W. P., Nieuwenhuizen, M. M., Meijer, E., in *Supramolecular Polymer Chemistry* **2011**, Wiley-VCH Verlag Weinheim, pp. 3.
[99] Sartorius, J., Schneider, H.-J., *Chem. Eur. J.* **1996**, *2*, 1446-1452.
[100] Wilson, A. J., *Soft Matter* **2007**, *3*, 409-425.
[101] Stadler, R., Lucca Freitas, L., *Colloid Polym. Sci.* **1986**, *264*, 773-778.
[102] Sijbesma, R. P., Beijer, F. H., Brunsveld, L., Folmer, B. J., Hirschberg, J. K., Lange, R. F., Lowe, J. K., Meijer, E., *Science* **1997**, *278*, 1601-1604.
[103] Beijer, F. H., Sijbesma, R. P., Kooijman, H., Spek, A. L., Meijer, E. W., *J. Am. Chem. Soc.* **1998**, *120*, 6761-6769.
[104] Folmer, B. J. B., Sijbesma, R. P., Versteegen, R. M., van der Rijt, J. A. J., Meijer, E. W., *Adv. Mater.* **2000**, *12*, 874-878.
[105] Hirschberg, J. H. K. K., Beijer, F. H., van Aert, H. A., Magusin, P. C. M. M., Sijbesma, R. P., Meijer, E. W., *Macromolecules* **1999**, *32*, 2696-2705.
[106] Sijbesma, R. P., Meijer, E. W., *Chem. Commun.* **2003**, 5-16.
[107] Rieth, L. R., Eaton, R. F., Coates, G. W., *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2153-2156.
[108] Elkins, C. L., Park, T., McKee, M. G., Long, T. E., *J. Polym. Sci. A Polym. Chem.* **2005**, *43*, 4618-4631.
[109] McKee, M. G., Elkins, C. L., Park, T., Long, T. E., *Macromolecules* **2005**, *38*, 6015-6023.
[110] Park, T., Zimmerman, S. C., Nakashima, S., *J. Am. Chem. Soc.* **2005**, *127*, 6520-6521.
[111] Park, T., Zimmerman, S. C., *J. Am. Chem. Soc.* **2006**, *128*, 14236-14237.
[112] Park, T., Zimmerman, S. C., *J. Am. Chem. Soc.* **2006**, *128*, 11582-11590.

[113] Nair, K. P., Breedveld, V., Weck, M., *Macromolecules* **2008**, *41*, 3429-3438.
[114] Colombani, O., Barioz, C., Bouteiller, L., Chanéac, C., Fompérie, L., Lortie, F., Montès, H., *Macromolecules* **2005**, *38*, 1752-1759.
[115] Falender, J., Yeh, G., Mark, J., *Macromolecules* **1979**, *12*, 1207-1209.
[116] Appel, E. A., Loh, X. J., Jones, S. T., Dreiss, C. A., Scherman, O. A., *Biomaterials* **2012**, *33*, 4646-4652.
[117] Dave, V., Tamagno, M., Focher, B., Marsano, E., *Macromolecules* **1995**, *28*, 3531-3539.
[118] Klemm, D., Heublein, B., Fink, H. P., Bohn, A., *Angew. Chem. Int. Ed. Engl.* **2005**, *44*, 3358-3393.
[119] Fatin-Rouge, N., Milon, A., Buffle, J., Goulet, R. R., Tessier, A., *J. Phys. Chem. B* **2003**, *107*, 12126-12137.
[120] Fernández, E., Hernández, R., Teresa Cuberes, M., Mijangos, C., López, D., *J. Polym. Sci. B Polym. Phys.* **2010**, *48*, 2403-2412.
[121] Kumachev, A., Greener, J., Tumarkin, E., Eiser, E., Zandstra, P. W., Kumacheva, E., *Biomaterials* **2011**, *32*, 1477-1483.
[122] Kartha, K., Srivastava, H., *Starch-Stärke* **1985**, *37*, 297-306.
[123] Kuniak, L., Marchessault, R., *Starch-Stärke* **1972**, *24*, 110-116.
[124] Xiao, C., *Starch - Stärke* **2013**, *65*, 82-88.
[125] Bock, L., *Ind. Eng. Chem.* **1937**, *29*, 985-987.
[126] Moon, R. J., Martini, A., Nairn, J., Simonsen, J., Youngblood, J., *Chem. Soc. Rev.* **2011**, *40*, 3941-3994.
[127] Nishio, Y., Haratani, T., Takahashi, T., Manley, R. S. J., *Macromolecules* **1989**, *22*, 2547-2549.
[128] Nishio, Y., Manley, R. J., *Macromolecules* **1988**, *21*, 1270-1277.
[129] Gupta, D., Tator, C. H., Shoichet, M. S., *Biomaterials* **2006**, *27*, 2370-2379.
[130] Edgar, K. J., Buchanan, C. M., Debenham, J. S., Rundquist, P. A., Seiler, B. D., Shelton, M. C., Tindall, D., *Prog. Polym. Sci.* **2001**, *26*, 1605-1688.
[131] Giri, T. K., Thakur, A., Alexander, A., Ajazuddin, Badwaik, H., Tripathi, D. K., *Acta Pharmaceutica Sinica B* **2012**, *2*, 439-449.
[132] Caicco, M. J., Zahir, T., Mothe, A. J., Ballios, B. G., Kihm, A. J., Tator, C. H., Shoichet, M. S., *J. Biomed. Mater. Res. A* **2013**, *101A*, 1472-1477.
[133] Wang, Y., Lapitsky, Y., Kang, C. E., Shoichet, M. S., *J. Control. Release* **2009**, *140*, 218-223.
[134] Chang, C., Lue, A., Zhang, L., *Macromol. Chem. Phys.* **2008**, *209*, 1266-1273.
[135] Dankers, P. Y. W., Hermans, T. M., Baughman, T. W., Kamikawa, Y., Kieltyka, R. E., Bastings, M. M. C., Janssen, H. M., Sommerdijk, N. A. J. M., Larsen, A., van Luyn, M. J. A., Bosman, A. W., Popa, E. R., Fytas, G., Meijer, E. W., *Adv. Mater.* **2012**, *24*, 2703-2709.
[136] Dankers, P. Y. W., van Luyn, M. J. A., Huizinga-van der Vlag, A., van Gemert, G. M. L., Petersen, A. H., Meijer, E. W., Janssen, H. M., Bosman, A. W., Popa, E. R., *Biomaterials* **2012**, *33*, 5144-5155.
[137] Kieltyka, R. E., Pape, A. C. H., Albertazzi, L., Nakano, Y., Bastings, M. M. C., Voets, I. K., Dankers, P. Y. W., Meijer, E. W., *J. Am. Chem. Soc.* **2013**, *135*, 11159-11164.
[138] Bastings, M. M. C., Koudstaal, S., Kieltyka, R. E., Nakano, Y., Pape, A. C. H., Feyen, D. A. M., van Slochteren, F. J., Doevendans, P. A., Sluijter, J. P. G., Meijer, E. W., Chamuleau, S. A. J., Dankers, P. Y. W., *Adv. Healthc. Mater.* **2014**, *3*, 70-78.
[139] Steed, J. W., Atwood, J. L., in *Supramolecular chemistry*, John Wiley & Sons, **2009**.
[140] Smith, D. K., *Nat. Chem.* **2010**, *2*, 162-163.
[141] Hofmeier, H., Schubert, U. S., *Macromol. Chem. Phys.* **2003**, *204*, 1391-1397.
[142] Meier, M. A. R., Schubert, U. S., *J. Polym. Sci. A Polym. Chem.* **2003**, *41*, 2964-2973.
[143] Ott, C., Ulbricht, C., Hoogenboom, R., Schubert, U. S., *Macromol. Rapid Commun.* **2012**, *33*, 556-561.
[144] Zhang, X., Pint, C. L., Lee, M. H., Schubert, B. E., Jamshidi, A., Takei, K., Ko, H., Gillies, A., Bardhan, R., Urban, J. J., Wu, M., Fearing, R., Javey, A., *Nano Lett.* **2011**, *11*, 3239-3244.

[145] Guillet, P., Mugemana, C., Stadler, F. J., Schubert, U. S., Fustin, C.-A., Bailly, C., Gohy, J.-F., *Soft Matter* **2009**, *5*, 3409-3411.
[146] Card, R. J., Neckers, D. C., *J. Am. Chem. Soc.* **1977**, *99*, 7733-7734.
[147] Furue, M., Sumi, K., Nozakura, S. I., *J. Polym. Sci. Polym. Lett. Ed.* **1982**, *20*, 291-295.
[148] Sumi, K., Furue, M., Nozakura, S. I., *J. Polym. Sci. A Polym. Chem.* **1984**, *22*, 3779-3788.
[149] Kokil, A., Yao, P., Weder, C., *Macromolecules* **2005**, *38*, 3800-3807.
[150] Nair, K. P., Breedveld, V., Weck, M., *Soft Matter* **2011**, *7*, 553-559.
[151] Beck, J. B., Rowan, S. J., *J. Am. Chem. Soc.* **2003**, *125*, 13922-13923.
[152] Beck, J. B., Ineman, J. M., Rowan, S. J., *Macromolecules* **2005**, *38*, 5060-5068.
[153] Iyer, P. K., Beck, J. B., Weder, C., Rowan, S. J., *Chem. Commun.* **2005**, 319-321.
[154] Rowan, S. J., Beck, J. B., *Faraday Discuss.* **2005**, *128*, 43-53.
[155] Weng, W., Beck, J. B., Jamieson, A. M., Rowan, S. J., *J. Am. Chem. Soc.* **2006**, *128*, 11663-11672.
[156] Zhao, Y., Beck, J. B., Rowan, S. J., Jamieson, A. M., *Macromolecules* **2004**, *37*, 3529-3531.
[157] Han, F. S., Higuchi, M., Kurth, D. G., *Adv. Mater.* **2007**, *19*, 3928-3931.
[158] Boontheekul, T., Kong, H.-J., Mooney, D. J., *Biomaterials* **2005**, *26*, 2455-2465.
[159] Dalsin, J. L., Hu, B.-H., Lee, B. P., Messersmith, P. B., *J. Am. Chem. Soc.* **2003**, *125*, 4253-4258.
[160] Fullenkamp, D. E., Rivera, J. G., Gong, Y.-k., Lau, K., He, L., Varshney, R., Messersmith, P. B., *Biomaterials* **2012**, *33*, 3783-3791.
[161] Holten-Andersen, N., Harrington, M. J., Birkedal, H., Lee, B. P., Messersmith, P. B., Lee, K. Y. C., Waite, J. H., *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 2651-2655.
[162] Lee, H., Scherer, N. F., Messersmith, P. B., *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12999-13003.
[163] Lin, Q., Gourdon, D., Sun, C., Holten-Andersen, N., Anderson, T. H., Waite, J. H., Israelachvili, J. N., *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 3782-3786.
[164] Wang, R., Both, S. K., Geven, M., Calucci, L., Forte, C., Dijkstra, P. J., Karperien, M., *Acta Biomater.* **2015**, *26*, 136-144.
[165] Dumitriu, S., Guttmann, H., Kahane, I., **1999**, US Patent 5,858,392.
[166] Hunt, J. N., Feldman, K. E., Lynd, N. A., Deek, J., Campos, L. M., Spruell, J. M., Hernandez, B. M., Kramer, E. J., Hawker, C. J., *Adv. Mater.* **2011**, *23*, 2327-2331.
[167] Zhang, T., Guo, Q., *Chem. Commun.* **2013**, *49*, 5076-5078.
[168] Zhang, T., Guo, Q., *Chem. Commun.* **2013**, *49*, 11803-11805.
[169] Ravi Kumar, M. N., *React. Funct. Polym.* **2000**, *46*, 1-27.
[170] Sashiwa, H., Aiba, S.-i., *Prog. Polym. Sci.* **2004**, *29*, 887-908.
[171] Patel, M., Mao, L., Wu, B., VandeVord, P. J., *J. Tissue Eng. Regen. Med.* **2007**, *1*, 360-367.
[172] Ishihara, M., Nakanishi, K., Ono, K., Sato, M., Kikuchi, M., Saito, Y., Yura, H., Matsui, T., Hattori, H., Uenoyama, M., *Biomaterials* **2002**, *23*, 833-840.
[173] Jimtaisong, A., Saewan, N., *Int. J. Cosmet. Sci.* **2014**, *36*, 12-21.
[174] Dumitriu, S., Magny, P., Montane, D., Vidal, P., Chornet, E., *J. Bioact. Compat. Polym.* **1994**, *9*, 184-209.
[175] Dumitriu, S., Chornet, E., Vidal, P., **1997**, US Patent 5,620,706.
[176] Dumitriu, S., Kahane, I., Guttmann, H., **1997**, US Patent 5,648,252.
[177] Chellat, F., Tabrizian, M., Dumitriu, S., Chornet, E., Magny, P., Rivard, C. H., Yahia, L. H., *J. Biomed. Mater. Res. A* **2000**, *51*, 107-116.
[178] Chellat, F., Tabrizian, M., Dumitriu, S., Chornet, E., Rivard, C.-H., Yahia, L., *J. Biomed. Mater. Res. A* **2000**, *53*, 592-599.
[179] Chornet, E., Ishizawa, C., Dumitriu, S., **2003**, US Patent 20,030,157,171 A1.
[180] Magnin, D., Lefebvre, J., Chornet, E., Dumitriu, S., *Carbohydr. Polym.* **2004**, *55*, 437-453.
[181] Pfister, L. A., Papaloïzos, M., Merkle, H. P., Gander, B., *J. Biomed. Mater. Res. A* **2007**, *80*, 932-937.
[182] Pfister, L. A., Alther, E., Papaloïzos, M., Merkle, H. P., Gander, B., *Eur. J. Pharm. Biopharm.* **2008**, *69*, 563-572.

[183] Wang, Q., Mynar, J. L., Yoshida, M., Lee, E., Lee, M., Okuro, K., Kinbara, K., Aida, T., *Nature* **2010**, *463*, 339-343.
[184] Tamesue, S., Ohtani, M., Yamada, K., Ishida, Y., Spruell, J. M., Lynd, N. A., Hawker, C. J., Aida, T., *J. Am. Chem. Soc.* **2013**, *135*, 15650-15655.
[185] Gong, J. P., Katsuyama, Y., Kurokawa, T., Osada, Y., *Adv. Mater.* **2003**, *15*, 1155-1158.
[186] Zhao, X., *Soft Matter* **2014**, *10*, 672-687.
[187] Sun, J.-Y., Zhao, X., Illeperuma, W. R., Chaudhuri, O., Oh, K. H., Mooney, D. J., Vlassak, J. J., Suo, Z., *Nature* **2012**, *489*, 133-136.
[188] Yoshida, R., Takahashi, T., Yamaguchi, T., Ichijo, H., *J. Am. Chem. Soc.* **1996**, *118*, 5134-5135.
[189] Yoshida, R., *Adv. Mater.* **2010**, *22*, 3463-3483.
[190] Vernon, L. B., Vernon, H. M., **1941**, US Patent 2,234,993.
[191] Osada, Y., Matsuda, A., *Nature* **1995**, *376*, 219.
[192] Liu, C., Qin, H., Mather, P., *J. Mater. Chem.* **2007**, *17*, 1543-1558.
[193] Han, Y., Bai, T., Liu, Y., Zhai, X., Liu, W., *Macromol. Rapid Commun.* **2012**, *33*, 225-231.
[194] Osada, Y., Okuzaki, H., Hori, H., *Nature* **1992**, *355*, 242-244.

7. Publications and Conference Contributions

Publications with Peer Review Process

1. T. Rossow, S. Hackelbusch, P. Van Assenbergh, S. Seiffert, *Polym. Chem.* **2013**, *4*, 2515–2527, *A modular construction kit for supramolecular polymer gels*.

2. S.Hackelbusch, T. Rossow, P. Van Assenbergh, S. Seiffert, *Macromolecules* **2013**, *46*, 6273–6286, *Chain dynamics in supramolecular polymer networks*.

3. S. Hackelbusch*, T. Rossow*, H. Becker, S. Seiffert, *Macromolecules* **2014**, *47*, 4028–4036, *Multiresponsive polymer hydrogels by orthogonal supramolecular chain crosslinking*. [* equal contribution]

4. S. Hackelbusch, T. Rossow, D. Steinhilber, D. A. Weitz, S. Seiffert, *Adv. Healthcare Mater.* **2015***, 4,* 1841–1848, "*Hybrid microgels with thermo-tunable elasticity for controllable cell confinement.*"

Publications without Peer Review Process

5. S. Hackelbusch, S. Seiffert, "*Polymeric supramolecular hydrogels as materials in medicine*" Ch.7 in X.J. Loh (Ed.) "In-situ gelling polymers for biomedical applications", Springer Science & Business Media, Singapore **2015**.

Conference Contributions

Poster Presentations

1. S. Hackelbusch, T. Rossow, S. Seiffert
   "A Modular Construction Kit for Supramolecular Polymer Gels" *Smart Polymers*, Mainz, Germany, 2012.

2. S. Hackelbusch, T. Rossow, S. Seiffert
   "A Modular Construction Kit for Supramolecular Polymer Gels" 14th *BPS*, Bayreuth, Germany, 2013.

8. Curriculum Vitae

For reasons of data protection, the curriculum vitae is not included in the online version.