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# Functional Polymer Gels by Click- and Supramolecular Chemistry

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# Contents

# List of Abbreviations and Symbols

| | |
|---|---|
| AAm | acrylamide |
| AFM | atomic force microscopy |
| ATRP | atom transfer radical polymerization |
| bpy | bipyridine |
| BIP | 2,6-bis(1-methylbenzimidazolyl)pyridine |
| BTP | 2,6-bis(1,2,3-triazol-4-yl)pyridine |
| CB[*n*] | cucurbit[*n*]uril |
| CuAAC | copper-catalyzed azide–alkyne cycloaddition |
| CD | cyclodextrin |
| DAN | 2,7-diamido-1,8-naphthyridine |
| DAP | diacyldiaminopyridine |
| DIFO | difluorinated cyclooctyne |
| DMSO | dimethyl sulfoxide |
| DOPA | dihydroxy-phenylalanine |
| DTT | dithiothreitol |
| ECM | extracellular matrix |
| EEGE | ethoxyethyl glycidyl ether |
| EPR | enhanced permeability and retention |
| FDA | US Food and Drug Administration |
| *G'* | elastic part of the shear modulus |
| *G''* | viscous part of the shear modulus |
| HEEDTA | hydroxyethyl ethylenediaminetriacetic acid |
| hMSC | human mesenchymal stem cell |
| hPG | hyperbranched polyglycerol |
| ITC | isothermal titration calorimetry |
| $K_{eq}$ | equilibrium binding constant |

| | |
|---|---|
| LCST | lower critical solution temperature |
| lPG | linear polyglycerol |
| MMP | matrix metalloproteinase |
| PAA | poly(acrylic acid) |
| PDMS | poly(dimethylsiloxane) |
| PEG | poly(ethylene glycol) |
| pNIPAAm | poly(*N*-isopropylacrylamide) |
| PPE | poly(*p*-phenylene ethynylene) |
| PVA | poly(vinyl alcohol) |
| PVP | poly(4-vinylpyridine) |
| py | pyridine |
| RAFT | reversible addition–fragmentation chain transfer polymerization |
| ROMBP | ring-opening multibranching polymerization |
| SAXS | small-angle X-ray scattering |
| SPAAC | strain-promoted azide–alkyne cycloaddition |
| $T_g$ | glass-transition temperature |
| tpy | terpyridine |
| UG | ureidoguanosine |
| UPy | 2-ureido-4-pyrimidinone |
| UV | ultraviolet |

# 1 Introduction

## 1.1 Covalent Polymer Gels

### 1.1.1 General

Polymer gels are solvent-swollen polymer networks that are insoluble due to a certain number of crosslinks, which entails a distinct three-dimensional structure. Polymer gels can be classified according to the nature of their crosslinks as covalent or supramolecular gels, according to their materials as synthetic or natural, according to their composition as homo- or copolymeric, and according to their size as nano-, micro-, or macrogels. If water is chosen as the swelling medium, polymer gels are commonly termed hydrogels and have gained extraordinary importance in life science applications.[1-3] In 1953, the work of Wichterle and Lim established the basis for this branch of research, when they described the synthesis of the first hydrogels for biomedical applications by copolymerization of 2-hydroxyethyl methacrylate with ethylene dimethacrylate.[4] From then on, the number of publications on hydrogels increased drastically, because they have reached outstanding potential in nanotechnology and pharmacy for the delivery of drugs,[5,6] peptides and proteins,[7,8] in everyday life as superabsorbers,[9] as well as in tissue engineering[10-14] and in regenerative medicine.[15,16] Hydrogels are also useful as three-dimensional synthetic extracellular matrixes (ECMs) for cell immobilization and transplantation, because they replace many functions of the native ECM, e.g., in view of organizing cells into a three-dimensional architecture, providing mechanical integrity to the new tissue, and offering a hydrated space for the diffusion of nutrients and metabolites to and from the cell.[14,17,18] This is due to their ability to absorb high amounts of water, their soft consistency, which is similar to that of native tissue, and due to their high permeability for low molecular weight substances. These features also contribute to their excellent biocompatibility, which has been defined as the *"ability to be in contact with a living system without producing an adverse effect."*[19]

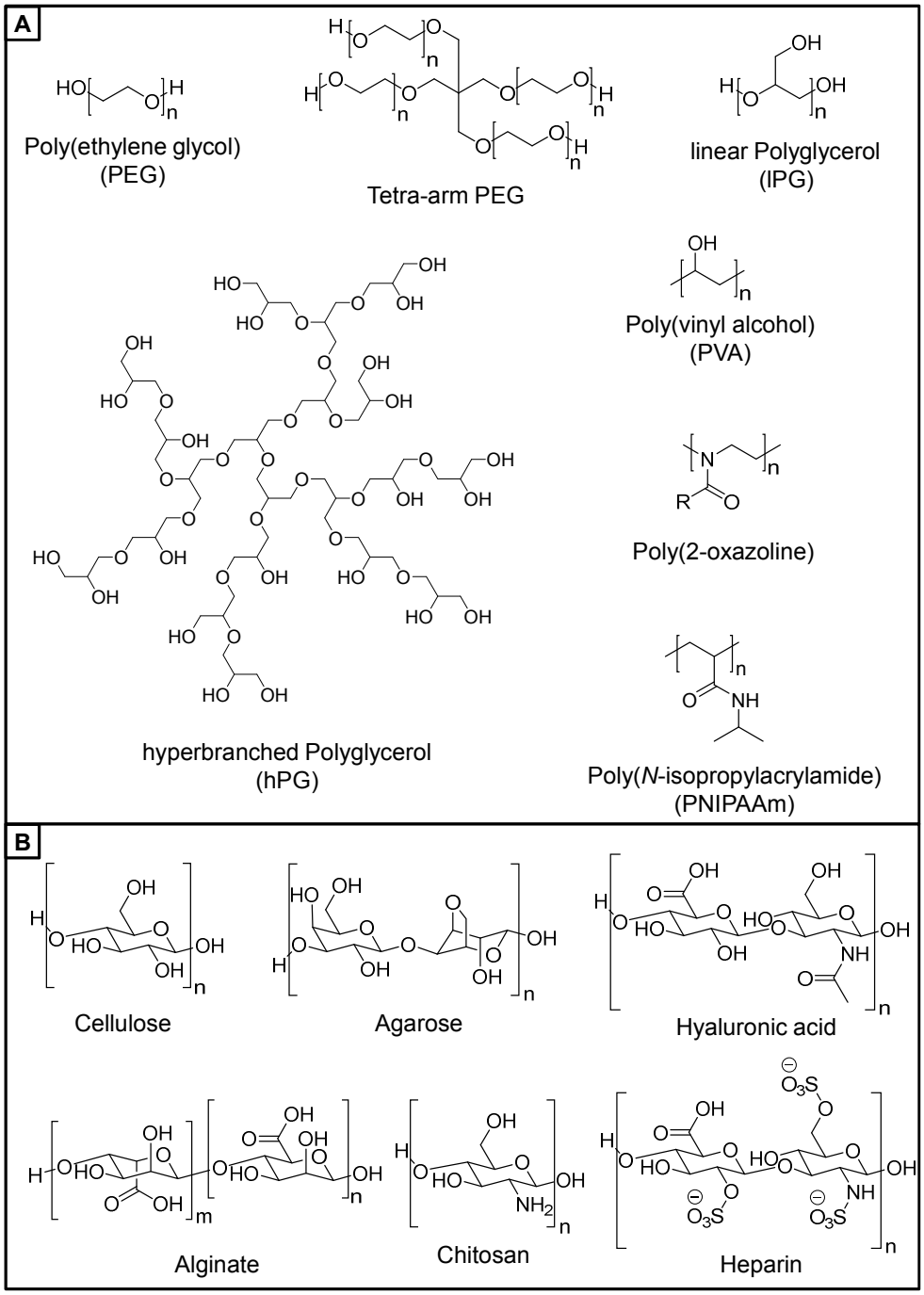
The following sections focus on the most frequently used synthetic precursor polymers and crosslinking chemistries for the preparation of functional micro- and macroscopic hydrogels with potential use for biological applications. The following survey also reveals some present challenges with respect to the preparation and formulation of such gels to overcome currently unsolved problems.

### 1.1.2 Macromonomers

Hydrogels can be formed from both synthetic and natural polymers. Commonly used biopolymers are alginate,[20] chitosan,[21] collagen, hyaluronic acid,[22] and fibrin.[23] Synthetic hydrogels can be formed

either by polymerization of monomers such as acrylamides,[24] acrylates, and methacrylates,[2] or by crosslinkage of macromolecular precursors, often referred to as macromonomers.

The crosslinkage of macromonomers affords the opportunity to exactly control the hydrogel composition and properties by the precursor concentration, molecular weight, and functionalization, along with the chemistry of crosslinking. An overview of commonly used synthetic and natural macromonomers for the formation of hydrogels is given in Scheme 1. Since natural polymers are typically crosslinked by physical interactions, they are discussed separately in Chapter 2.




**Scheme 1.** Overview of commonly used (A) synthetic and (B) natural macromonomers for the formation of hydrogels.

### 1.1.2.1 Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) is a commercially available polyether backbone polymer that is soluble in a variety of different solvents including water, methanol, ethanol, toluene, and dichloromethane. It can be prepared in a well controllable way by anionic polymerization of ethylene oxide entailing a wide range of accessible molecular weights from 300 g $mol^{-1}$ to 10,000,000 g $mol^{-1}$ along with narrow molecular weight distributions. By appropriate choice of the initiator, monofunctional, homo- and heterobifunctional linear PEG, as well as PEG dendrimers and multi-arm PEGs can be prepared.[25] Postmodification of hydroxy-terminated PEG provides access to a large variety of functionalities, such as acrylates,[26] methacrylates,[27] vinyl sulfones,[28] thiols,[29] maleimides,[30] alkynes,[31] azides,[31] amines,[32] carboxylates, and active esters.[33] These functional groups can serve to form three-dimensional crosslinked polymer networks by using linear PEG-acrylates or -methacrylates and applying free-radical chemistry[34,35] or by mixing functional multiarm-PEGs with complementary functional linear PEGs.[26] The resulting polymer networks often display polydisperse mesh sizes, along with structural imperfections such as loops and dangling chains entailing low mechanical strength of the gels.[36-39] However, by using two functional tetra-arm PEGs of complementary reactivity, high-strength hydrogels with ideally homogenous network structures, called 'model networks',[40] can be formed, as shown by Sakai, Shibayama, and co-workers.[33,41,42] This is because each macromonomer has four end groups reacting with each other in an alternating fashion, thereby avoiding self-reaction.

PEGs are the most frequently used synthetic macromonomers in the biological area, because PEGs and their resulting hydrogels have been believed to be non-immunogenic, non-toxic, and inert to protein and cell interactions.[25] As a result, PEG, PEG-hydrogels, and PEG-containing formulations have been approved by the U.S. Food and Drug Administration (FDA) and thereafter widely been used for the encapsulation of proteins and cells.[43] Furthermore, PEG–protein conjugates are promising therapeutic agents, because PEGylation overcomes protein degradability caused by proteolytic enzymes.[44] In addition, renal clearance is lowered, and the circulating half-times in the blood are prolonged by increase of the molar mass of the proteins by conjugation to the PEG. However, recently, the formation of antibodies upon administration of a PEGylated protein has been observed,[45] which questions the immunogenicity of PEGs and necessitates the search for alternatives.

### 1.1.2.2 Polyglycerol

Polyglycerols are structurally similar to PEG and exhibit analogous physical properties, but offer multiple hydroxy functionalities that allow postmodification while the polymer remains water-soluble.[46] By this means, reactive functionalities including acrylates,[47] methacrylates,[48] alkynes,[49]

azides,[49] carboxylates,[50] and amines[51] can be introduced, allowing bioactive molecules to be coupled to polyglycerol carriers.[52,53] Because polyglycerol is known to be protein and cell resistant[54,55] and also less cytotoxic than the FDA-approved PEG,[56,57] it is well suited as hydrogel building block for biological applications. Polyglycerols of different structure such as perfect dendrimers,[58] as well as hyperbranched[59] (hPG) and linear analogues[60] (lPG) have been prepared.

Hyperbranched polymers are not built as perfectly as dendrimers and exhibit polydisperse molecular weights, but in advantage to dendrimers, they can be synthesized in a single step. Traditionally, hPGs are synthesized by ring-opening polymerization of 3-hydroxypropylene oxide (glycidol), a commercially available and highly reactive hydroxy epoxide.[61-63] In 1999, Sunder et al. introduced the ring-opening multibranching polymerization (ROMBP) of glycidol.[59] The use of a partially deprotonated triol as alkoxide initiator and slow addition of the monomer leads to hyperbranched polyols with a well-defined polyether structure, along with narrow polydispersities and molecular weights between 1.25 kDa and 6.5 kDa. Meanwhile, the mechanism of the polymerization process has been well understood and can be modeled, thereby affording precise control over the molecular weight and degree of branching in hPG syntheses.[64,65] Moreover, Brooks and co-workers reported the synthesis of hPG with very high molecular weights of up to 700 kDa using emulsion polymerization.[66,67]

Linear polyglycerols can be prepared by polymerization of protected glycidols, e.g., of ethoxyethyl glycidyl ether (EEGE), which can be easily prepared by reaction of glycidol with ethyl vinyl ether[68] and later on be easily deprotected at acidic conditions. Several polymerization methods for EEGE have been developed, comprising both anionic and coordinated types;[69-71] however, either multimodal or broad molecular weight distributions along with low molecular weights of only 30,000 g $mol^{-1}$ (protected form) have been obtained. Recently, Carlotti et al. introduced the synthesis of lPGs with high molar masses by a monomer-activated anionic polymerization.[72] The authors used tetraalkyl ammonium salts as initiators and triisobutylaluminum as activator for the polymerization. By this means, lPGs of molar masses of up to 85,000 g $mol^{-1}$ (protected form) along with narrow polydispersities can be obtained.

Hennink et al. used methacrylated hPG to prepare hydrogels by free-radical crosslinking.[48] These hydrogels have high storage moduli and only show limited swelling in comparison to PEG hydrogels, which was attributed to the rather rigid network of intermolecular crosslinked hPG precursors. The hydrogel properties are controllable by varying the hPG concentration or the degree of hPG functionalization with methacrylate groups. Polyglycerol nanogels have been fabricated using the miniemulsion polymerization technique by either crosslinking existing hPGs in a “click” reaction[49] (Chapter 1.1.3) or by employing acid-catalyzed polyaddition of glycerol to trisglycidyl glycerol ether.[73]

#### 1.1.2.3 Poly(2-oxazoline)s

Poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx) are promising polymeric precursors for biological applications and have been widely investigated as drug delivery systems.[74,75] They can be prepared by the living cationic ring-opening polymerization of cyclic 2-oxazolines along with narrow polydispersities,[74] but the molecular weights achieved by this method are limited to 40,000 g $mol^{-1}$.[76] There are several reports on the preparation of hydrogels using poly(2-oxazoline)s precursors,[77,78] e.g., by radical polymerization of vinyl end-functionalized PMeOx bis-macromonomers.[79]

Poly(2-oxazoline)s exhibit blood-circulation times and organ distribution similar to PEG, along with just low cytotoxicity and immunogenicity.[80,81] Moreover, they have found to be stable at physiological conditions for a long time and therefore were believed to be useful in long-term in-vivo applications,[82] in which PEG might be degraded oxidatively.[83] However, very recent results indicate oxidative degradation of poly(2-oxazoline)s.[84]

#### 1.1.2.4 Poly(*N*-isopropylacrylamide)

Since its first synthesis in 1956,[85] poly(*N*-isopropylacrylamide) (pNIPAAm) has become famous and important for its temperature-responsiveness in aqueous media.[86] Upon heating, pNIPAAm changes its polymer–solvent interaction from being hydrophilic to being hydrophobic, entailing a dramatic polymer-coil volume shrinkage. The corresponding temperature is known as lower critical solution temperature (LCST), and in dependence on the polymer microstructure, it lies between 30 °C and 35 °C, close to body temperature. As a result, pNIPAAm gels have been widely exploited in tissue engineering[87,88] and drug delivery applications,[89,90] although its biocompatibility is highly controversial and has not been proven yet.[91] Ingber et. al encapsulated mesenchymal stem cells into thermo-responsive pNIPAAm gels to mechanically induce tooth differentiation in vitro and in vivo by promoting stem cell compaction.[92]

Typically, pNIPAAm is synthesized from *N*-isopropylacrylamide, which is commercially available, by free-radical initiation in organic solvents or by redox initiation in aqueous media.[86] As a result of the absence of reactive side groups in the polymer backbone, postmodification of pNIPAAm is not possible. Hence, pNIPAAm hydrogels are often prepared by copolymerization with *N*,*N*′-methylenebisacrylamide.[93] By using chain-transfer reagents and free-radical polymerization, one chain-end of pNIPAAm can be functionalized.[94,95] When difunctional comonomers are used, however, crosslinkable groups are introducible along the pNIPAAm chain; subsequent macromonomer crosslinking triggers gelation.[86] By this means, Weitz and Seiffert prepared thermo-responsive pNIPAAm microgel capsules using photocrosslinkable pNIPAAm-*co*-acrylamide macromonomers.[96] In

a similar approach, also Janus microgels have been fabricated from functional pNIPAAm precursor polymers.[97]

Besides free-radical polymerization, pNIPAAm can be prepared by controlled radical polymerization such as atom transfer radical polymerization (ATRP) or reversible addition–fragmentation chain transfer polymerization (RAFT) giving rise to narrow molecular weight distributions. In addition, these methods allow the polymer to be functionalized with reactive groups on both chain ends.[98,99] Applying RAFT polymerization, Whittaker and co-workers synthesized diazide-terminated pNIPAAm and crosslinked a tetraacetylene via copper-catalyzed azide–alkyne cycloaddition to a highly regular model network.[100] Peng et al. were also able to prepare pNIPAAm model networks by using dithiol and diallyl-modified pNIPAAm precursors and by crosslinking them in a photoinitiated thiol–ene click reaction.[101]

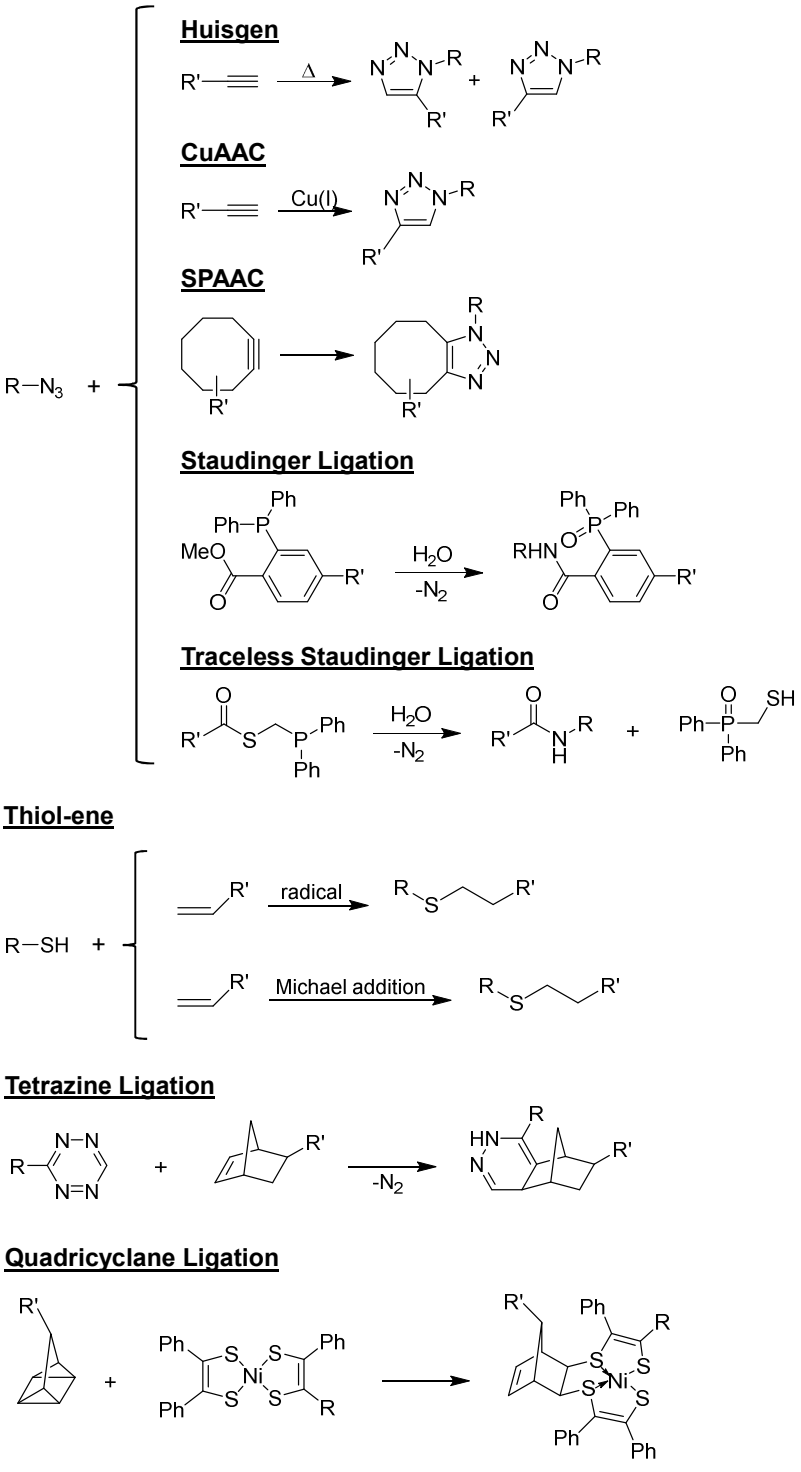
### 1.1.3 Click Chemistry

The crosslinkage of macromonomers to form polymer gels can occur by many different reaction types, but if the gels are anticipated to serve for the encapsulation of sensitive biomolecules or cells, not all of such reactions have turned out to be equally effective. Most widely used is the free-radical crosslinking of acrylates or methacrylates, because these functionalities can be easily introduced to hydroxy-functionalized polymers via ester-bonding; by this means, even bifunctional linear polymers can be radically crosslinked to form polymer networks. Besides radical initiation by heat or redox-reactions, photoinitiation by exposure to UV light in the presence of photoinitiators has been employed extensively for the synthesis of macro-, micro-, and nanoscopic hydrogels.[48,102,103] Although this method was applied successfully for the encapsulation of biological specimen,[104,105] photoinitiators and UV irradiation are potentially cytotoxic. Metters et al. observed protein inactivation and incomplete protein release after photoencapsulation,[106] and Dhert et al. observed adverse effects of photopolymerization on the viability and proliferation of multipotent stromal cells.[107] As a consequence, mild crosslinking reactions are required that are orthogonal to the functional groups of the biomolecules, non-cytotoxic, and fast at 37 °C.

One class of chemistry that fulfills these requirements is termed ‘bioorthogonal’ and was introduced by Carolyn R. Bertozzi in 2003 as chemical reactions that can occur inside of living systems without interfering with biological processes.[108-110] For this purpose, the substrates of bioorthogonal reactions must react selectively with each other at fast rates under physiological conditions, and they must be inert to the functionalities found in vivo.

Bioorthogonal chemistry is a subclass of ‘click chemistry’[111] that was defined by K. Barry Sharpless in 2001 as follows: *“The reaction must be modular, wide in scope, give very high yields, generate only*

*inoffensive by-products that can be removed by nonchromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily available starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation."*[112] An overview of commonly used and recently developed click reactions is given in Scheme 2.



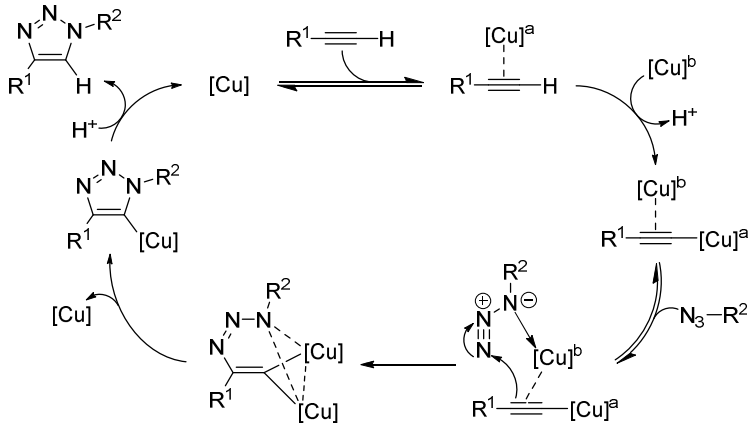

**Scheme 2.** Overview of commonly used and recently developed click reactions.

### 1.1.3.1 Azide–Alkyne Huisgen Cycloaddition

Azides react as 1,3-dipoles when converted with alkynes in a concerted [3+2] cycloaddition, leading to aromatic triazole products as proposed by Rolf Huisgen in the 1950s, when he introduced his concept of 1,3-dipolar cycloadditions.[113] This reaction is the prime example of click chemistry and was referred to as the *"cream of the crop"* of it by K. Barry Sharpless.[112] The particular attractivity of the alkyne and azide functionalities as complementary coupling partners is based on the ease of their synthesis, their kinetic stability, and their tolerance to a wide variety of functional groups and reaction conditions. Although this reaction has been served to form hydrogels by crosslinkage of azide- and alkyne-functionalized macromonomers,[49] the high temperatures or pressures that are required to promote this reaction are not compatible with living systems. To address this problem, Kiser and Clark developed a Huisgen cycloaddition for hydrogel formation that works at physiological temperature by using electron-deficient alkynes; [114] the reaction rates, however, are relatively slow.

### 1.1.3.2 Copper-Catalyzed Azide–Alkyne Cycloaddition

Independent of each other, the groups of Sharpless[115] and Meldal[116] discovered a dramatic rate acceleration of the azide–alkyne coupling along with the selective formation of 1,4-disubstituted 1,2,3-triazoles under copper(I) catalysis. This is because the alkyne is activated by the formation of a copper acetylide toward the reaction with azides; as a result, the reaction even occurs at room temperature. The mechanism of this reaction, which is termed copper-catalyzed azide–alkyne cycloaddition (CuAAC), has remained difficult to establish and is still subject to ongoing research.[117-119] Very recently, Fokin et al. figured out that two chemically equivalent copper atoms are involved together in the triazole formation.[120] The proposed mechanism for the CuAAC is shown in Scheme 3.




**Scheme 3.** Proposed mechanism for the copper-catalyzed azide–alkyne cycloaddition.[120]

The CuAAC has become the most widely used click reaction in organic synthesis,[121] polymer chemistry,[122] materials chemistry,[123] chemical biology,[124] and in medicinal[125] and pharmaceutical chemistries.[126] Its potential for hydrogel preparation could be demonstrated, for example, by Hawker et al.,[127] who synthesized well-defined PEG hydrogels by crosslinking alkyne-terminated tetra-arm PEG and diazide-terminated PEG by CuAAC. Yang and co-workers synthesized PEG–peptide hydrogels by crosslinking diazide-terminated peptides and alkyne-terminated tetra-arm PEGs.[128] The obtained hydrogels exhibit improved mechanical properties in comparison to photochemically-crosslinked PEG gels.

Because azides and alkynes are not natively present in organisms and react fast and selectively under physiological conditions in the presence of a copper catalyst, the CuAAC is sometimes also referred as bioorthogonal.[109] The use of the CuAAC, however, is limited in living systems due to the cytotoxicity of copper(I). Mammalian cells can survive below 500 µM of copper(I) for 1 hour,[129] but considerable cell death occurs when optimized CuAAC conditions that require 1 mM copper(I) are employed.[130] As a consequence, the strain-promoted azide–alkyne cycloaddition (SPAAC), which is a click reaction that has the same orthogonality as the CuAAC but which can refrain from cytotoxic copper, has been developed.

#### 1.1.3.3 Strain-Promoted Azide–Alkyne Cycloaddition

In 1961, Krebs and Wittig observed that the reaction between cyclooctyne, the smallest stable cycloalkyne, and phenyl azide proceeds extremely fast and yields a single product, the triazole.[131] In comparison to the reaction of linear alkynes with azides, the activation barrier for the cycloaddition of cyclooctynes with azides is lowered due to the massive bond-angle distortion of the alkyne to 163°,[132] accounting for a ring strain of almost 18 kcal $mol^{-1}$.[133] As a result, the strain-promoted azide–alkyne cycloaddition occurs at room temperature without need for a catalyst. In 2004, Bertozzi et al. initially employed this reaction toward in-vitro labeling of biomolecules at physiological conditions.[109,134] An azide-functionalized glycoprotein was incubated with a cyclooctyne-modified biotin-containing fragment. Biotinylation was observed for the azide-functionalized glycoprotein, whereas control samples of the native protein lacking azides showed no labeling. As a further control, a similar reaction was carried out using biotin functionalized with a terminal alkyne. This experiment showed no glycoprotein labeling in the absence of copper, whereas copper catalysis resulted in facile labeling of the azido-modified glycoprotein.

Taking this powerful methodology to the next level, Bertozzi and co-workers performed in-vivo labeling studies of living cells.[134,135] After incorporation of azide-functionalized sialic acid into the cells, they were treated with cyclooctyne-modified biotin. Subsequent staining of the cells with

FITCavidin, which can bind to biotin with a high degree of affinity and specificity, showed fluorescence with an intensity that depends on the prior dose of cyclooctyne. In comparison to CuAAC, SPAAC exhibits considerably slower kinetics, which is a drawback for several applications. Thus, Bertozzi and co-workers developed a cyclooctyne derivative bearing electron-withdrawing fluorine atoms next to the alkyne. While one fluorine atom resulted in a four-fold rate increase,[136] geminal difluoro atoms afforded a sixty-fold increase.[135] This difluorinated cyclooctyne (DIFO) exhibited comparable kinetics to CuAAC in biomolecule labeling experiments and was therefore termed as "copper-free click chemistry". DIFO–fluorophore conjugates have proven to be exceptionally useful reagents for the imaging of azide-labeled biomolecules within complex biological systems such as living cells,[135] C. elegans,[129] and zebrafish embryos.[137] Anseth et al. introduced this copper-free click chemistry to biomaterial formation, when they crosslinked tetra-arm PEG-azide with bis-DIFO-functionalized polypeptides in the presence of living cells to generate fibroblast-laden hydrogels with excellent cell viabilities higher than 90%.[138] On the basis of these results, SPAAC was considered as absolutely bioorthogonal; the spontaneous addition of thiols to cyclooctyne, however, has been observed recently.[139] While these results suggest that the sensitivity of fluorescence assays based on SPACC is limited when thiol-containing proteins or cells are present, several other experiments have revealed that the reactivity and kinetics of cyclooctynes toward azides are much higher than toward thiols,[140] thereby maintaining the suitability of SPAAC for biomolecule and cell encapsulation.

The synthetic effort for DIFO derivatives limits the broad application of SPAAC in biolabeling and biomaterial applications. Recently, van Delft et al. established an alternative synthetic route to highly reactive cyclooctynes that drastically reduces the number of reaction steps to four entailing an excellent overall yield of almost 50%.[141]

#### 1.1.3.4 Thiol–Ene Chemistry

The highly efficient hydrothiolation of carbon–carbon double bonds, or simply "enes", by thiols is known since 1905.[142] In particular, two types of thiol reactions have gained attention during the last decades, because they provide many of the attributes of click chemistry. One type is the thiol–ene free-radical addition to electron-rich/electron-poor carbon–carbon double bonds; another type is the thiol-Michael addition to electron-deficient carbon–carbon double bonds such as vinyl sulfones, acrylates, and maleimides.[143-145] Both reactions lead to quantitative yields of just one single product, respectively, without any necessity for subsequent purification. In addition, both reactions exhibit high rates with just little or no need for additional catalysis, and they are insensitive to ambient oxygen or water. Moreover, there is an enormous range of both thiols and enes commercially available. As a result, the thiol–ene radical and thiol-Michael addition, respectively, are routinely

referred to as 'thiol click reactions' in the literature.[146,147] The exceptional versatility of these reactions has stimulated their application in the preparation of high performance polymer networks,[148] in optics,[149] sensing,[150] and biomedicine.[151,152] Whereas the thiol–ene radical reaction is commonly induced by UV light in the presence of a photoinitiator, the thiol-Michael addition can proceed under physiological conditions without need of any catalyst for initiation. Although the thiol–ene radical reaction has been used in the presence of cells with apparently no detrimental effect on their viability, photoinitiators and UV irradiation are potentially cytotoxic.[107] The thiol-Michael addition, however, is not completely bioorthogonal either: Even though Hubbel et al. have proven the thiol-Michael addition to be selective versus biological amines,[153] it can interfere with thiol-containing biomolecules or cells;[109] this is a problem of the strain-promoted azide–alkyne cycloaddition, too, but with less extent. An advantage of the thiol-Michael addition to SPAAC is the easy synthetic access to its substrates with high yields. Its applicability for hydrogel formation in biological applications has been demonstrated several times despite the potential side reaction with thiol-containing biomolecules.[154,155]

#### 1.1.3.5 Other Click Reactions

Another type of bioorthogonal reaction is the Staudinger ligation[156] of azides and triarylphosphines, which is a modification of the Staudinger reduction of azides with triphenylphosphine.[157] By introduction of an ester group to one of the phosphine aryl substituents, Bertozzi et al.[156] could link triarylphosphine to organic azides via an amide bond, accompanied by phosphine oxide formation. In contrast, when using the classical Staudinger reaction, no covalent linkage between triphenylphosphine and azides is observed after hydrolysis. In a following work, Bertozzi and co-workers modified this reaction to cleave the phosphine oxide group after amide formation and therefore called this reaction 'traceless' Staudinger ligation.[158] A serious drawback of the Staudinger ligation is its very slow kinetics, as well as phosphine oxidation by air,[109] both largely restricting its use for hydrogel formation to just a few examples.[159,160]

In contrast, tetrazine ligation, which is an inverse-demand Diels–Alder reaction of *trans*-cyclooctene and tetrazine with subsequent elimination of nitrogen in a retro-Diels–Alder reaction, has been reported to be extremely fast.[161] Hilderbrand and co-workers used this reaction for coupling of fluorescent tetrazine derivatives to norbornene tagged living cells.[162] As a side reaction in living organisms, a reaction of tetrazines with linear alkenes of the cell membrane can occur, but the kinetics of this reaction is much slower.[163] Hence, tetrazine ligation can be termed bioorthogonal and used for labeling living cells[162] and encapsulating cells into hydrogels.[164]

Recently, Bertozzi et al. developed a new type of bioorthogonal chemistry, the quadricyclane ligation.[165] In this ligation, a highly strained hydrocarbon quadricyclane and nickel bis(dithiolene)s react with each other. The kinetics is similar to that of bioorthogonal reactions of azides, and the reaction is compatible and orthogonal to the strain-promoted azide–alkyne cycloaddition. As a result, both reactions can be carried out in one pot.[165]

### 1.1.4 Microgels for Cell Encapsulation

Microgels are hydrogel particles with micrometer-scale dimensions.[166,167] The concept of microgels has already been introduced 50 years ago by Chang and co-workers based on the idea to protect encapsulated materials from the external environment.[168] Today, microgels find multiple applications in drug delivery,[169] biosensing,[170] catalysis,[171] and regenerative medicine.[172] They are particularly useful for the encapsulation of living cells, because the microgel matrix can mimic the natural ECM, and because microgel particles can be handled by syringes and pipettes and are injectable.[173] By this means, cells can be studied and manipulated in a 3D environment,[174] in which they behave very different from cells on rigid 2D substrates.[175,176] Moreover, microgel particles are suited to study the impact of matrix elasticity on cellular behavior, which is known to be the main stimulus for stem cell fate in the human body.[177-179] In addition, these particles can be used to create larger tissue scaffolds by particle self-assembly, as shown by Khademhosseini and co-workers.[173,180]

In addition to these different areas of service in fundamental cellular research, cell-laden microgels are promising candidates for practical applications such as those in tissue engineering and therapeutics. This is because cell-laden microgels can be transplanted to patients without any need of immune-suppressing medicals, because leucocytes, antibodies, and enzymes, which can cause immune defense, are unable to pass the polymeric matrix due to their high molecular weights, as illustrated in Figure 1.[181] In contrast, the continuous supply of the encapsulated cells with smaller molecules such as nutrients, oxygen, metabolites, hormones, peptides, and small proteins during cell proliferation is possible.[182]

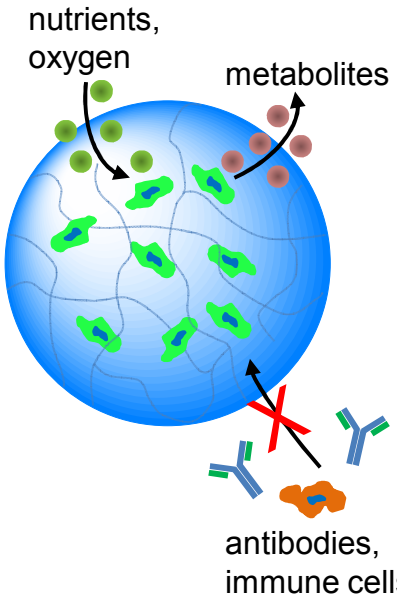



**Figure 1.** Cell encapsulation into microgels. Nutrients, oxygen, and metabolites can diffuse across the membrane and penetrate into the microgel interior, whereas antibodies and immune cells are excluded from penetrating the microgel.[181]

Several in-vivo studies in animals[183,184] and even in humans[185] have confirmed the potential of microencapsulated cells for therapeutic treatment of diabetes, but have coincidently shown the necessity for the development of new polymeric matrixes. The materials often caused a foreign-body response that resulted in biofilm formation on the capsules, along with necrosis of encapsulated cells. Fundamental research of microencapsulated cells also requires new materials that offer the on-demand-release of the encapsulated cells after culturing to allow for detailed cellular studies using high-throughput methods such as flow cytometry. For this purpose, highly biocompatible, bioinert, and tunable synthetic polymeric precursors such as polyglycerol and PEG should be used along with reversible and responsive chemical or physical crosslinks.

### 1.1.5 Methods for Microgel Preparation

Microgels can be prepared by different techniques depending on whether they are formed in the 0.1–10 µm colloidal domain or in the 10–1000 µm above-colloidal domain.[186] For the synthesis of colloidal microgels, precipitation polymerization is the most frequently used method.[187] In this approach, monomers and crosslinkers are polymerized at conditions wherein which the forming polymer chains are not soluble and precipitate after a short period of free chain growth. Then, the precipitating chains agglomerate and serve as nuclei that are grown by further addition of monomer and oligoradicals.

Above-colloidal microgels can be prepared by use of different emulsion templating techniques: in photolithography, a pattern is transferred from a mask onto a substrate via UV illumination, whereas

in soft lithography, 2D patterns and 3D structures are created by using elastomeric materials such as stamps and molds. By employing these techniques in combination with free-radical photopolymerization, Hennink and co-workers prepared microgels from methacrylated hPG, as shown in Figure 2.[103] However, the limited degree of swelling of pure polyglycerol gels prevents them from being used in several biological applications, including cell encapsulation.

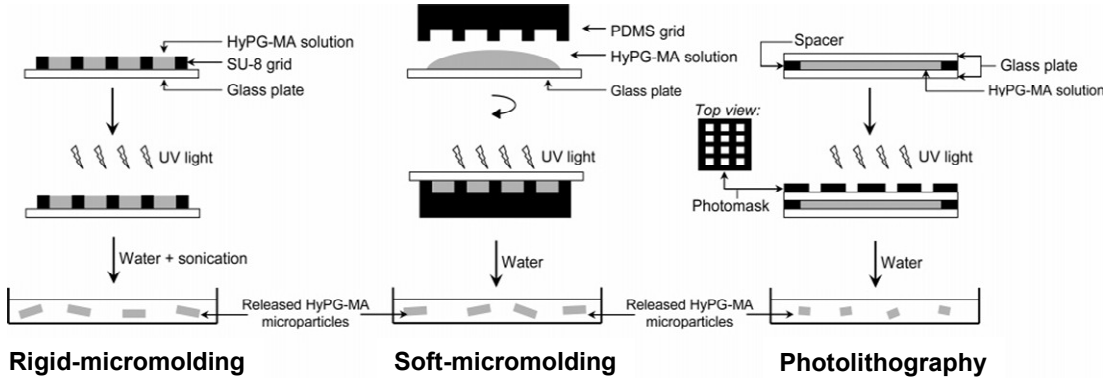



**Figure 2.** Process of fabrication of microparticles that consist of methacrylated hPG using rigid and soft micromolding as well as photolithography. Reprinted with permission from ref. 103. Copyright 2007 American Chemical Society.

Another versatile and powerful technique to prepare above-colloidal microgels is droplet-based microfluidics.[188-191] In this approach, monodisperse emulsion droplets are formed and used as templates for the microgel synthesis. The size, shape, and monodispersity of the microparticles can be adjusted by controlling the size, shape, and monodispersity of the pre-microgel droplets. For droplet microfluidics, either glass capillary devices or devices made by lithography techniques, commonly consisting of poly(dimethylsiloxane) (PDMS),[192] can be used. Glass capillary microfluidic devices consist of coaxial assemblies of glass capillary tubes with cylindrical or square cross-sections glued onto glass slides. Their wettability is easily and precisely controllable by a quick surface reaction with an appropriate surface modifier. By this means, robust resistance to organic solvents is created allowing the formation of truly three-dimensional flows. In this point, glass microcapillary-based devices are superior to those molded from PDMS, because PDMS is incompatible with most organic solvents. The attractive feature of PDMS devices with aqueous solution, e.g., in cell encapsulation, is the ability to fabricate highly complex flow channels for upstream adjustment of the fluid streams and downstream manipulation of the droplets. Moreover, lithography makes mass-production of these devices possible; once a given type of photomask is designed, a large number of devices can be replicated from it.[193]

In a flow focusing microfluidic device made by lithography, a stream of a polymer or polymer-precursor solution (dispersed phase) is created in the central channel, and its periodic breakup is induced by the shear force of a second, immiscible fluid (continuous phase) introduced to the side

channels, as illustrated in Figure 3. By this means, monodisperse, micrometer-sized droplets are formed. Subsequent droplet solidification retains their uniform size and shape and yields polymer particles with a precisely controlled morphology.

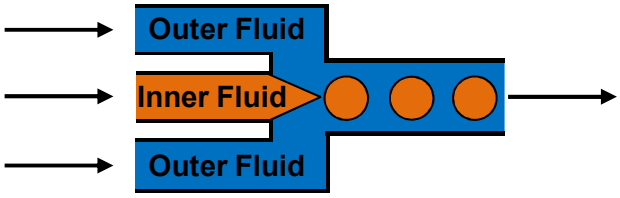



**Figure 3.** Schematic of a simple flow-focusing microfluidic device.

In a variant application of the microfluidic method, fibroblast-laden microgel particles were prepared from photopolymerized PEG-macromonomers by Doyle and co-workers using stop-flow-lithography (single-phase microfluidic polymerization).[194] In this approach, a solution of PEG-diacrylate was flown through a microfluidic device and then partly solidified by pulsed UV light irradiation through a transparency mask, thereby periodically producing arrays of individual particles. As a result, cell-laden microgels of different shapes have been synthesized. The cell viabilities of the encapsulated fibroblasts were determined to be 70%, whereby radicals and UV-light might have detrimental effects on the cell viabilities.

To avoid UV-irradiation, Haag, Seiffert, and colleagues prepared yeast cell-laden microgels by free-radical crosslinking using thermally-generated radicals.[47] Because these microgel particles consist of hyperbranched polyglycerol and PEG, the degree of microgel swelling could be increased and the elastic modulus decreased in comparison to pure hPG macro- and microgels,[48,103] thereby making these particles more favorable for cell encapsulation.[195] However, despite the biocompatibility and favorable elasticity of the hPG–PEG polymer matrix, the presence of free radicals during its formation turned out to be detrimental for the cell viability, which was determined to be ~30% only.

### 1.1.6 Stimuli-Responsive Gels

Stimuli-responsive hydrogels, often also called smart hydrogels, are able to change their properties according to their environment.[196] Such materials are responsive to temperature,[90] pH,[197] light,[198] enzymes,[28] and electric[199] or magnetic fields,[200] whereupon they respond in various ways, such as through degradation,[201] change of their shape[202] or volume (swelling or shrinking),[90] or variation of their color or transparency.[203] By this means, encapsulated ingredients can be released, which is important for several applications. In drug delivery, an encapsulated drug can be transported to a target site in a state of nanogel encapsulation, be accumulated by the EPR-effect (enhanced

permeability and retention) in tumor tissue,[204,205] and then released by application of a suitable stimulus.[206] Alternatively, in tissue engineering, stimuli-responsive gels can be used for temporary cell encapsulation, allowing the cells to be studied and manipulated during encapsulation and then isolated and harvested on demand by decomposition of the hydrogel scaffolds.[13] Furthermore, degradation of the hydrogel matrix is known to have direct impact on cell differentiation,[207] proliferation,[208] and migration.[209] For this purpose, either stimuli-responsive polymers[210] such as pNIPAAm (Chapter 1.1.2.4) can be used, or responsive functionalities can be incorporated into the polymer network. By the latter method, Hubbell et al. prepared enzyme-degradable fibroblast-laden hydrogels by thiol-Michael crosslinking of vinyl sulfone-functionalized tetra-arm PEG and bis-cystein peptides that are sensitive to matrix metalloproteinases (MMPs), an enzyme family involved in tissue development and remodeling.[211] Enzymatic degradation is well suited if the stimulus is induced from the inside of the hydrogel, as in the case of encapsulated cells. If the stimulus is initiated externally by addition of enzymes, the degradation kinetics is difficult to control, because it depends on the enzyme diffusion into the hydrogel to reach the cleavable sites. As an external trigger, Murphy et al. used the base-catalyzed ester hydrolysis.[212] For this purpose, PEG-diacrylate and dithiothreitol (DTT) were reacted by step-growth polymerization to form acrylate-terminated $(\text{-PEG-DTT-})_n$ chains, and subsequent photocrosslinking resulted in hydrogel-network formation. The hydrolytic lability of this gel under physiological pH of 7.4 is due to the presence of a thioether bond proximal to an acrylate ester bond, thereby enhancing its reactivity toward nucleophilic hydroxyl anions in the primary step of base-catalyzed ester hydrolysis. The authors could demonstrate that hydrogel degradability has a significant effect on the behavior of human mesenchymal stem cells (hMSCs) encapsulated within these hydrogels, since enhanced network degradability has resulted in enhanced hMSC viability and spreading during in-vitro culture.

As an expedient alternative to chemical or thermodynamic stimulation, light is an excellent external stimulus to actuate sensitive-microgel response, since its intensity and the space and duration of illumination can be exactly controlled; however, encapsulated cells are not actively involved in the degradation process. To make use of these advantages, Anseth and co-workers prepared photodegradable stem cell-laden hydrogels by free-radical crosslinking using a nitrobenzyl-ether as photodegradable functionality.[213] Upon irradiation with light, cells could be released on demand, making this system promising as a 3D cell culture platform.

Another useful stimulus to induce internally and externally triggered hydrogel degradation is hydrolysis at acidic pH, because protons are known to play a major role in cellular communication,[214] and they show an unusually high diffusion rate due to their small size and due to their rapid moving through water according to the Grotthuss mechanism.[215,216] By this means, encapsulated cells can actively degrade the hydrogel matrix from the inside, but such gels can also be degraded from the

outside, which is of particular use if they are implanted into tumor or inflamed tissue that both possess a decreased pH of just 6.5, in comparison to healthy tissue with pH 7.4.[217] The degradation kinetics only depends on the stability/lability of the responsive functional group and is therefore controllable by its selection. So far, acid-cleavable hydrogels have been prepared by free-radical crosslinking[218,219] and CuAAC,[54] but not by bioorthogonal click chemistry allowing living cells to be encapsulated. Moreover, bioorthogonal click chemistry has not been combined with droplet-based microfluidics to create microgel particles.

An alternative to stimuli-responsive covalent hydrogels, the responsiveness of which is induced by the selective choice of functional groups, is introduced by supramolecular polymer gels. They are intrinsically responsive due to their transient crosslinks; thus, they are promising materials for a wide range of applications.

## 1.2 Supramolecular Polymer Networks and Gels

### 1.2.1 Supramolecular Interactions

"*Supramolecular chemistry may be defined as 'chemistry beyond the molecule', bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces*." — this definition has been given by J.-M. Lehn in 1987,[220] when he received the Nobel prize together with C. J. Pedersen and D. J. Cram for their work on host–guest chemistry. Since then, supramolecular chemistry has gained increasing attention and developed to an important, broad, and active field of research.[221-223] Today, research on supramolecular chemistry may be subdivided into several categories dealing with host–guest complexes,[224-226] self-assembled architectures,[227,228] supramolecular polymers,[229-233] supramolecular gels,[234-237] and supramolecular polymer networks.[238,239]

The term supramolecular polymer refers to a polymer built of monomeric units associated through directional non-covalent physical interactions. Supramolecular gels typically consist of low molecular weight precursors that self-assemble to three-dimensional networks through non-covalent interactions; these materials are often brittle and hard to customize. In contrast, supramolecular polymer networks consist of covalently jointed macromolecular building blocks (polymers) that are functionalized with motifs that can bind to each other through non-covalent interactions such as hydrogen bonding,[240-242] transition metal complexation,[230,243,244] hydrophobic interaction,[245] ionic attraction,[229] or π–π stacking,[246-248] serving to assemble the polymer chains to a network. Non-covalent interactions strongly vary in their strength, as shown in Figure 4. As a result, supramolecular polymer materials can be tuned to exhibit different mechanical properties by custom use of these interactions for polymer crosslinking.

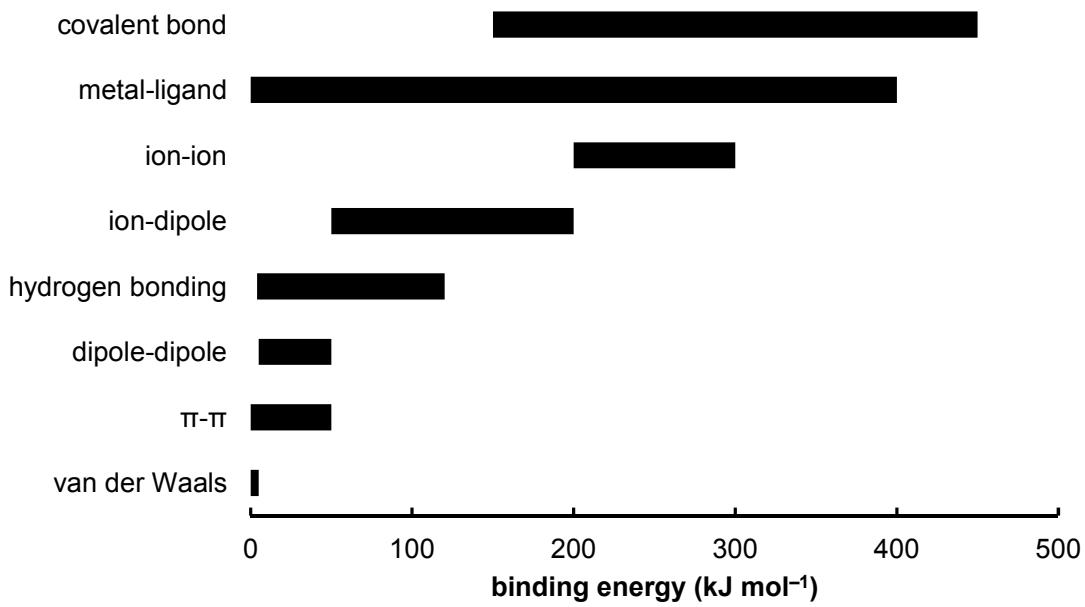



**Figure 4.** Overview of the most important non-covalent interactions along with their binding strength in comparison to covalent bonds.[249]

Supramolecular polymer networks combine the characteristics of chemical and physical networks, because they can be tailored to specific needs through the use of macromolecular building blocks. While forming strong materials at favorable conditions, they are easily decrosslinked at other conditions. Physically associating motifs can be divided into those that associate with one other in a self-complementary fashion or those that require a different complementary motif to associate with in a hetero-complementary fashion. The latter approach is useful to get access to more sophisticated materials, because in this approach, the strength of crosslinking is tunable by various complementary motifs. Different design principles can serve to build up such supramolecular networks, as illustrated in Figure 5. In one principle, linear chains functionalized with supramolecular linkable motifs at both chain ends are crosslinked if these motifs form associative nodes with a functionality greater than two (Figure 5A). In a second principle, systems that form supramolecular linear chains can be crosslinked by entanglement of the polymer chains or by phase separation through lateral interactions of the transient crosslinks, including stacking, clustering, or crystallization (Figure 5B). In a third principle, the supramolecular motifs are attached as side chains to a polymer backbone, resulting in polymer crosslinking even if the supramolecular motifs assemble in just a bivalent fashion. If hetero-complementary motifs are used, crosslinking is achievable by addition of low-molecular weight crosslinking agents to the supramolecular crosslinkable polymers (Figure 5C) or by using a second polymer functionalized with a complementary supramolecular motif (Figure 5D). The supramolecular motifs can be introduced to the side chains after synthesis of the polymer backbone

in a post-polymerization step. As an alternative, monomers that contain the supramolecular motifs beforehand can be polymerized in a suitable chain- or step-growth process.

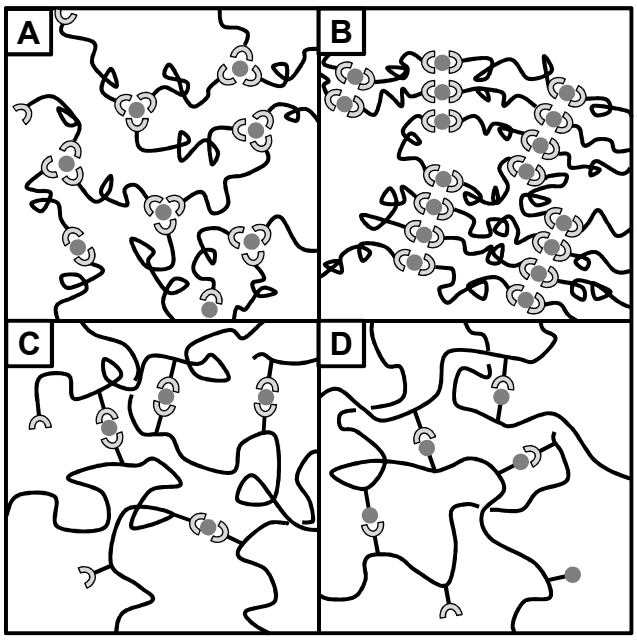



**Figure 5.** Overview of different design principles to prepare supramolecular polymer networks by hetero-complementary interactions. Crosslinking of end-capped linear chains by (A) associative nodes with a functionality higher than two or by (B) additional lateral chain interactions. As an alternative, side-chain functionalized polymer chains can associate by (C) low-molecular weight crosslinkers or (D) mutual hetero-complementary polymer–polymer binding.

Due to their transient and reversible crosslinking, supramolecular polymer networks are responsive[223] to external stimuli such as variation of temperature,[250] pH,[251] polarity of the solvent,[252] redox reactions,[253] and competitive ligation.[254] This tunability makes them useful for a plethora of applications. They can be used as drug delivery systems[255] and matrixes in tissue engineering,[256] because drugs and cells can be encapsulated and protected within these materials and afterwards be released on demand at a targeted site of action. Furthermore, supramolecular polymer networks often have self-healing properties,[257,258] because after rupture, supramolecular bonds can reassociate when brought into contact, thereby healing the material. In addition, the combination of supramolecular and covalent crosslinking gives rise to shape-memory materials.[259]

Many different polymeric precursors can be used to prepare supramolecular polymer networks, including synthetic polymers, natural polymers, and hybrids of both. In approaches that serve to derive fundamental physical-chemical understanding of supramolecular polymer networks, mostly synthetic precursors are used, because chemical modification allows them to be tailored as desired. When it comes to further tailoring of supramolecular polymer materials for practical applications in the biological area, a popular class of precursor polymers are those that are water-soluble, including poly(ethylene glycol),[260,261] poly(vinyl alcohol) (PVA),[262-264] and polyglycerol.[265-267] However, several

other synthetic precursors are soluble in organic solvents only. In addition, many supramolecular polymer networks are labile in water, because many binding motifs do not form interactions strong enough to withstand competitive hydrogen bonding. Hence, a popular alternative to fully synthetic supramolecular polymer gels are natural polymer gels,[10] such as those based on alginate,[268-271] gelatin,[272] or chitosan,[273] which can form hydrogels even without chemical modification. These materials are biocompatible, bioavailable, biodegradable, and cheap, which makes them ideal candidates for life science applications.[274] However, natural polymers also have disadvantages:[275] they differ in their composition from batch to batch since they are harvested from living organisms,[276,277] the production of large volumes of natural polymers is limited, and they cannot be tailored on the demand of different applications since their properties are determined by the species that produce them.[278] As a result, the combination of synthetic and natural polymeric precursors in hybrid networks often presents an excellent compromise.

The following chapters describe the preparation and characterization of supramolecular polymer networks, particularly emphasizing on their physical-chemical features with regard to the type and strength of physical chain crosslinking and the resulting macroscopic material properties. Furthermore, recent work on the formation and characterization of supramolecular hydrogels based on synthetic and natural precursors, respectively, is summarized with focus on their application and potential in biomedicine.

### 1.2.2 Supramolecular Polymer Networks and Organogels

#### 1.2.2.1 Hydrogen Bonding

Hydrogen bonding plays a crucial role in many biological processes such as DNA base pairing, ligand–receptor binding, enzyme catalysis, and protein folding; it has also become the most widely used non-covalent interaction for the synthesis of supramolecular polymers and reversibly crosslinked polymer networks.[279-284] This is not due to the binding strength of hydrogen bonds, which is just 4–120 kJ $mol^{-1}$ (Figure 4), but due to their strong directionality and versatility.

After the description of weak interactions between molecules containing hydroxyl groups by Nernst in 1892,[285] the actual term ‘hydrogen bond’ was first introduced by Bernal and Huggins in 1935.[286,287] Hydrogen bonds connect atoms X and Y that have electronegativities larger than that of hydrogen. The XH group is generally referred to as the ‘proton donor’ (D), whereas Y is called the ‘proton acceptor’ (A).[279] An increase in the dipole moment of the X–H bond and the electron lone pair on atom Y entails an increase in the hydrogen-bonding strength. For the strength of hydrogen-bonded complexes, however, less the strength of the single hydrogen bond but rather the number of hydrogen bonds within a hydrogen-bonding motif is crucial. When acting together in a cooperative

fashion, hydrogen bonds become much stronger than their simple numerical sum. The binding constant of the DNA base pair guanine–cytosine (Figure 6A), which contains three hydrogen bonds, is therefore two to three orders of magnitude higher than that of the adenine–thymine complex that contains just two hydrogen bonds.[281] In the guanine–cytosine complex, not only the higher number of primary hydrogen bonds plays an important role, but also secondary interactions arising due to the particular arrangement of neighboring donor and acceptor sites, as shown in Figure 6A. Complexes between the ADA–DAD motifs exhibit an association constant of around $10^2$ $M^{-1}$ in chloroform, whereas DAA–ADD complexes exhibit binding constants of $10^4$ $M^{-1}$.[288] AAA–DDD arrays even have association constants higher than $10^5$ $M^{-1}$. Jorgenson et al.[289,290] have attributed this effect to differences in secondary interactions between these motifs. Diagonally opposed sites electrostatically repel each other when they are of the same kind, whereas contrary sites attract each other. Hence, the ADA–DAD complex has four repulsive secondary interactions, the DAA–ADD complex has two repulsive and two attractive interactions, and the AAA–DDD complex has four attracting interactions, as visualized in Figure 6B.

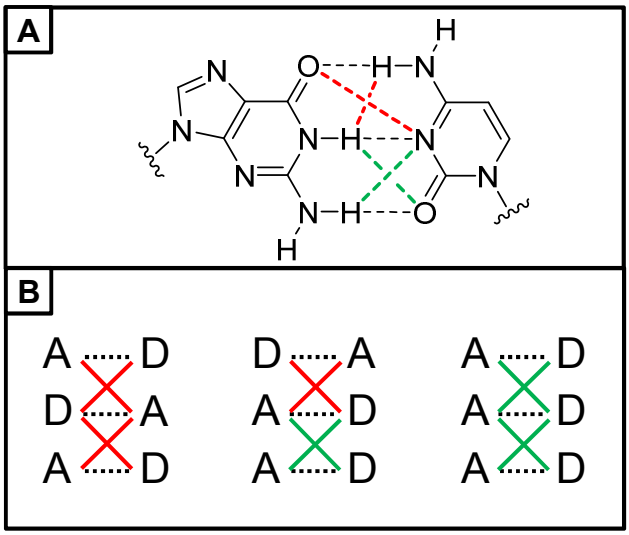
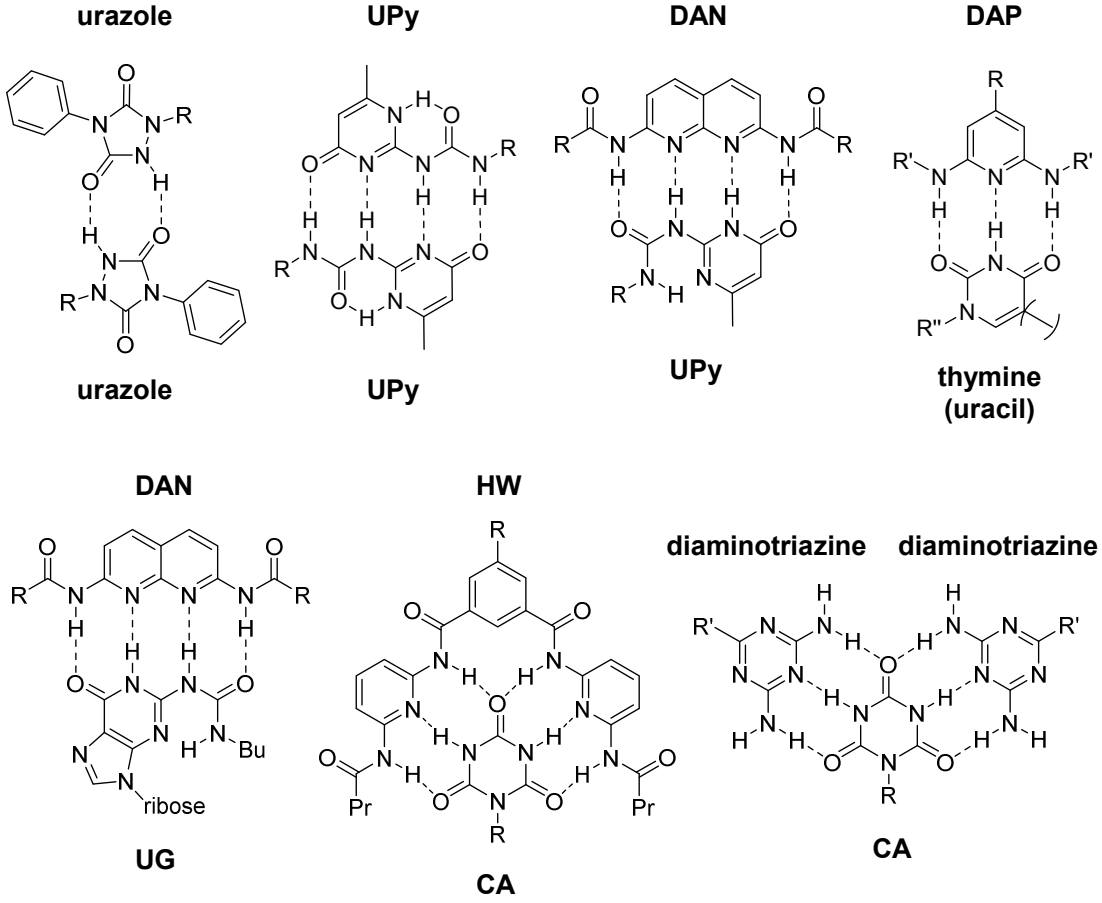



**Figure 6.** Secondary hydrogen-bonding interactions. Attractive interactions are marked green, whereas repulsive interactions are marked red. (A) Guanine–cytosine complex in the DNA-strand. (B) Possible secondary interactions in different triple hydrogen-bonding motifs.

Calculations by Schneider reveal that the arrangement of several hydrogen bonds can be related by a linear correlation in which primary interactions contribute –8.0 kJ $mol^{-1}$ to the complex stability, whereas secondary attractive or repulsive interactions contribute ± 2.9 kJ $mol^{-1}$, respectively.[291] In addition to secondary interactions, preorganization, intramolecular hydrogen bonding, tautomerization, and electronic substituent effects of the hydrogen-bonding motifs significantly contribute to the cooperative effect.[281] For preorganization, a rigid aromatic framework is often used that presents multiple hydrogen-bonding sites wherein which an entropy cost has to be paid only for

the formation of the first hydrogen bond. In case of amides, however, which are able to rotate and often stay in the preferred *trans*-confirmation in the uncomplexed form, the amide bond has to rotate to the *cis*-confirmation for complex formation and needs to be fixed in this position, which costs entropy. An overview of hydrogen-bonding motifs discussed in this chapter is given in Scheme 4.




**Scheme 4.** Overview of hydrogen-bonding motifs discussed in this thesis.

In a pioneering application, the formation of thermoplastic elastomers crosslinked by hydrogen bonding was explored by Stadler and co-workers in 1986.[292-294] In this work, unpolar polybutadienes with narrow molecular weight distributions were modified with urazole side groups. Hydrogen bonding between the highly polar urazole groups gives rise to the formation of thermo-reversible elastomeric networks. The rheological properties of these networks were investigated in the melt. No rubbery elastic equilibrium network modulus is observed due to the fragility of the transient hydrogen-bonding linkages. At low frequencies, Newtonian flow is predominant due to the same reason. Hence, although this approach has shown the potential of hydrogen bonding to form supramolecular polymer networks, it remained impossible to transfer this concept to the formation

of strong organogels for more than ten subsequent years, which was due to the difficulty to prepare stronger hydrogen-bonding motifs.

In 1997, Meijer and co-workers developed easy access to derivatives of 2-ureido-4-pyrimidinone (UPy) and demonstrated strong dimerization of these compounds, with $K_{eq} > 10^6$ $M^{-1}$ in chloroform.[295] This is due to assembly of a self-complementary DDAA array of four hydrogen bonds,[296] preorganized by an intramolecular hydrogen bond and therefore markedly stabilized.[295,297] Meijer et al. attached these UPy motifs to both chain ends of polyethylene polymers forming viscous solutions in chloroform;[296] the viscosity is highly concentration- and temperature-dependent and can be described by the Cates model for reversibly breaking wormlike micelles[298-300] that is also adaptable to supramolecular polymers above the overlap concentration.[301-305] To exclusively ascribe the observed viscosity to the formation of linear supramolecular polymers, a monofunctional UPy motif acting as a chain stopper was added; as a result, a dramatic decrease in viscosity was observed. To further study reversibly crosslinked networks, three UPy motifs were attached to poly(ethylene oxide-*co*-propylene oxide) polymers.[296,306] In oscillatory shear rheology under bulk conditions at 30 °C, this compound shows a frequency-dependent transition from purely viscous to viscoelastic behavior with increasing frequency, whereupon *G'* exceeds *G''* and a plateau modulus of $5 \cdot 10^5$ Pa is apparent. This plateau modulus is six times higher than that of the same copolymer when it is crosslinked covalently. This observation is referred to the reversibility of the hydrogen bonding that allows the polymer chains to assemble into a more dense, thermodynamically determined network, whereas the covalent crosslinks are irreversible and form a kinetically determined network. The supramolecular crosslinked networks show no additional stabilization such as crystallization or other kinds of phase separation. Based on this observation, Meijer and co-workers extended their investigations of UPy-crosslinked supramolecular polymer networks in view of their solution behavior in chloroform and tetrahydrofuran.[306] The viscosity of the solutions is significantly affected by the polarity of the solvent, and again, addition of a chain stopper leads to a dramatic decrease in viscosity. These experiments demonstrate that a supramolecular crosslinked, reversible polymer network is formed in solution.

In the following, Meijer, Sijbesma, and co-workers developed new synthetic strategies capable of coupling UPy moieties to polysiloxanes[307,308] and to a variety of hydroxy-telechelic polymers such as polyethers, polyesters, and polycarbonates.[309] OH-telechelic poly(ethylene-*co*-butylene), which is almost completely amorphous and apolar and therefore increases the strength of hydrogen bonds, was functionalized with UPy.[309] Whereas OH-telechelic poly(ethylene-*co*-butylene) is a viscous liquid (Figure 7A), the same polymer functionalized with hydrogen-bonding units is an elastic solid (Figure 7B). In dynamic oscillatory shear measurements, a broad rubbery elastic plateau with a storage modulus of about $10^6$ Pa is observed at high frequencies. Thus, this linear supramolecular

polymer exhibits mechanical properties even stronger than those in the previously discussed supramolecular UPy-based networks.[296,306] This finding suggests that in addition to hydrogen bonding within the polymer chains, interchain interactions such as physical crosslinks are likely to be present; however, the transparent appearance of the material indicates that large clusters of hydrogen-bonded units are absent. An explanation for this finding could be the formation of urethane lateral hydrogen-bonding interactions, as shown in Figure 7C and 7D. Reinvestigation of this material in a later study by atomic force microscopy (AFM) indeed confirmed the presence of fibrillar aggregates at room temperature (Figure 7E).[310] By using urea groups instead of the urethanes as linkers to attach the UPy to the polymer, the lateral interactions are increased, as evidenced through the formation of longer and more densely packed fibers by AFM, and the mechanical properties of the materials are further enhanced.

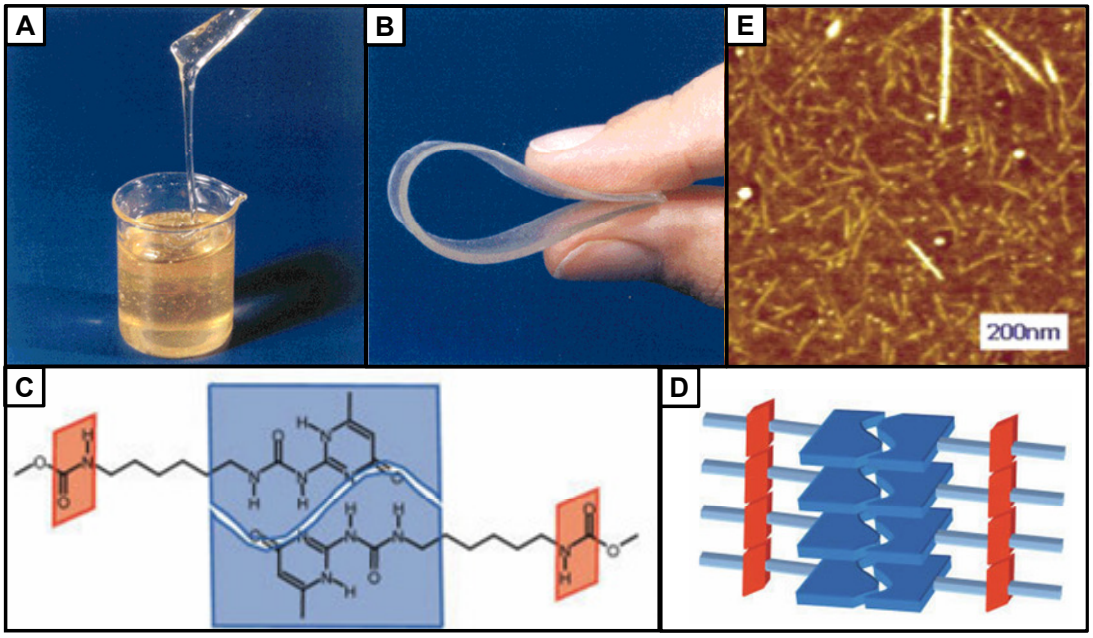



**Figure 7.** Hydrogen-bonded thermoplastic elastomer based on self-complementary interactions of ureido-pyrimidinone (UPy). (A) Poly(ethylene-*co*-butylene) with OH end groups. (B) Poly(ethylene-*co*-butylene) functionalized with UPy. (C),(D) Lateral interactions through π–π stacking of UPy and hydrogen bonding of urethane linkers. (E) AFM image of nanofibers formed through these lateral interactions. Reprinted with permission from ref. 308–310. Copyright 2000, 2006, 2008 WILEY-VCH Verlag GmbH, Wiley Periodicals, Inc., and American Chemical Society.

Other research groups have started to incorporate UPy motifs as side chains into higher molecular weight polymers via random copolymerization of UPy-functionalized alkene[311] or methacrylate monomers.[312-314] Coates et al. prepared UPy side-chain functionalized polyolefines by co-polymerizing 1-hexene and alkene-functionalized UPy derivatives using late transition metal Ziegler–Natta catalysts.[311] By this means, copolymers with molecular weights between 33 and 104 kg $mol^{-1}$ were obtained containing 2 mol% of UPy, along with narrow molecular weight distributions. At

concentrations higher than 20 g $L^{-1}$ these copolymers form organogels in toluene. Addition of a monofunctional UPy derivative leads to complete decrosslinking, suggesting that individual sets of hydrogen-bonded dimers are the predominant source of network formation; stacks or clusters of UPy moieties would not be completely broken down by the presence of monofunctional UPy. Kramer and Hawker used another strategy and prepared random copolymers of poly(*n*-butyl acrylate)s via controlled radical polymerization and introduced UPy moieties by post-polymerization functionalization.[315] Through this synthetic strategy, both the molecular weight and the content of the UPy monomer are excellently controllable, and very high UPy monomer contents of up to 15 mol% are achievable along with low polydispersities. Moreover, triblock copolymers were synthesized containing a homopolymer midblock and random copolymer endblocks, effectively concentrating the hydrogen-bonding groups near the chain ends. This leads to dramatic changes in the dynamic properties of these materials in bulk: they show strongly increased effective bond lifetimes in comparison to the random copolymers, and as a result, elastomeric behavior is observed on much longer time scales.

Besides self-complementary UPy, which is one of the most important and extensively studied hydrogen-bonding motifs, several hetero-complementary arrays of multiple hydrogen bonds serving to form supramolecular polymer networks have been developed, as also shown in Scheme 4.[281,316] Zimmerman and co-workers synthesized polystyrene with 2,7-diamido-1,8-naphthyridine (DAN) in the side chains, which is a ADDA hydrogen-bonding array, and also poly(butylmethacrylate) with ureidoguanosine (UG) in the side chains, which is a DAAD array.[317-319] Polystyrene and poly(butylmethacrylate) themselves are immiscible and do not form blends, as shown in Figure 8A. Mixed solutions of the DAN- and UG-functionalized polymers, however, give transparent films upon drying as a result of strong quadruple hydrogen bonding between DAN and UG ($K_{eq}$ = 3 · $10^8$ $M^{-1}$ in chloroform), as demonstrated in Figure 8B. Additionally, poly(butylmethacrylate) was side-chain functionalized with UPy, which is able to interact with DAN by quadruple hydrogen bonding in the form of its ADDA tautomer.[295,320] Mixing of this polymer with the DAN-functionalized polystyrene yields viscous solutions in chloroform; however, the viscosity exhibits lower values than in the case of DAN–UG mixtures, most likely due to competitive UPy-self-association.

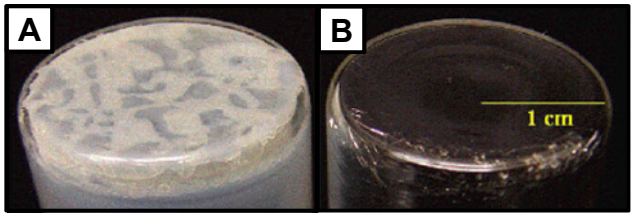



**Figure 8.** Films of intrinsically immiscible polymers with and without promotion by supramolecular linking. (A) Heterogeneous film of plain poly(butylmethacrylate) and polystyrene functionalized with 2,7-diamido-1,8-naphthyridine (DAN). (B) Transparent blend of poly(butylmethacrylate) functionalized with ureidoguanosine (UG) and polystyrene functionalized with DAN, thereby enabling quadruple hydrogen bonding of DAN and UG to facilitate polymer mixing. Reprinted with permission from ref. 319. Copyright 2006 American Chemical Society.

Weck and co-workers prepared polymers side-chain functionalized with cyanuric acid motifs by ring-opening metathesis polymerization of norbornene-based cyanuric acid and spacer monomers.[321] To these, low molecular weight ditopic crosslinking agents based on 2,4-diaminotriazine, which form three-point hydrogen bonding with cyanuric acid, or a Hamilton wedge, which forms six-point hydrogen bonding with cyanuric acid, were added to achieve crosslinking in 1-chloronaphthalene. The extent of crosslinking is controllable by the amount of the crosslinking agent. Whereas addition of the Hamilton wedge crosslinking agent leads to highly viscous fluids, the 2,4-diaminotriazine crosslinking agent produces highly viscoelastic gels, despite just weak triple hydrogen-bonding interactions in contrast to the stronger six-point hydrogen bonding of the Hamilton wedge. The authors hypothesized that this is due to a higher degree of network connectivity in the 2,4-diaminotriazine system, since *two* diaminotriazine motifs can complex to one cyanuric acid moiety. Hence, it is not only the strength of the hydrogen bond chain linkage, but also the assembly of these crosslinks and the network microstructure that determines the macroscopic mechanical network properties. Similar clustering and micellization of hetero-complementary hydrogen-bonding motifs has also been observed by Binder et al., who studied telechelic polyisobutylenes with thymine and diaminotriazine end groups.[322] Clustering and stacking of supramolecular crosslinks often enforces supramolecular networks rather than making them weaker,[233,306,322-325] which stands in marked contrast to covalently crosslinked networks, wherein which nanostructural network heterogeneity is assumed to entail weaker materials.[36-39,326,327]

Later on, Weck et al. extended their studies on hydrogen-bonding side-chain polymers by norbornene-based precursor polymers containing different hydrogen-bonding motifs.[328] That way, selective de- and recrosslinking is achievable by competitive hydrogen bonding. Besides thermal responsiveness, this competitive binding allows the mechanical properties of the networks to be controlled over a broad range, from low viscous liquids to elastic gels.

The potential of hydrogen bonding for the preparation of polymersomes has been demonstrated by Rotello et al., who prepared functional polystyrene copolymers and attached

diacyldiaminopyridine (DAP) or thymine derivatives to their side chains.[329] By mixing equal volumes of both polymers at low concentrations (3 g $L^{-1}$ in chloroform), formation of vesicular aggregates (3 µm in diameter) is observed due to complementary three-point hydrogen bonding between diacyldiaminopyridine and thymine. When polystyrene containing a higher DAP content (50 mol%) is dissolved in chloroform, discrete microspheres are created by self-complementary hydrogen bonding of DAP.[330] Addition of thymine-functionalized polystyrene leads to morphology transition into vesicles by hetero-complementary hydrogen bond formation, as visualized in Figure 9. By the same way, mono- and multivalent guests that can undergo competitive hydrogen bonding can be specifically incorporated in this type of polymersomes.[331] In an alternative approach, diacyldiaminopyridine-functionalized polystyrenes have been crosslinked by a series of linear bis-thymines, giving rise to filled spherical microspheres rather than vesicles.[332] These aggregates dissociate at 50 °C, but reform upon cooling. When the heating–cooling cycle is repeated several times, particles with a narrower size distribution are obtained. To extend the formation of polymersomes to other polymer types, Rotello et al. prepared diacyldiaminopyridine and uracil side-chain functionalized polynorbornene copolymers.[333] Again, polymersomes with diameters of several micrometers were formed through three-point hydrogen bonding in chloroform; however, these aggregates are only metastable, and after 2 h of aging, macroscopic organogels are obtained.

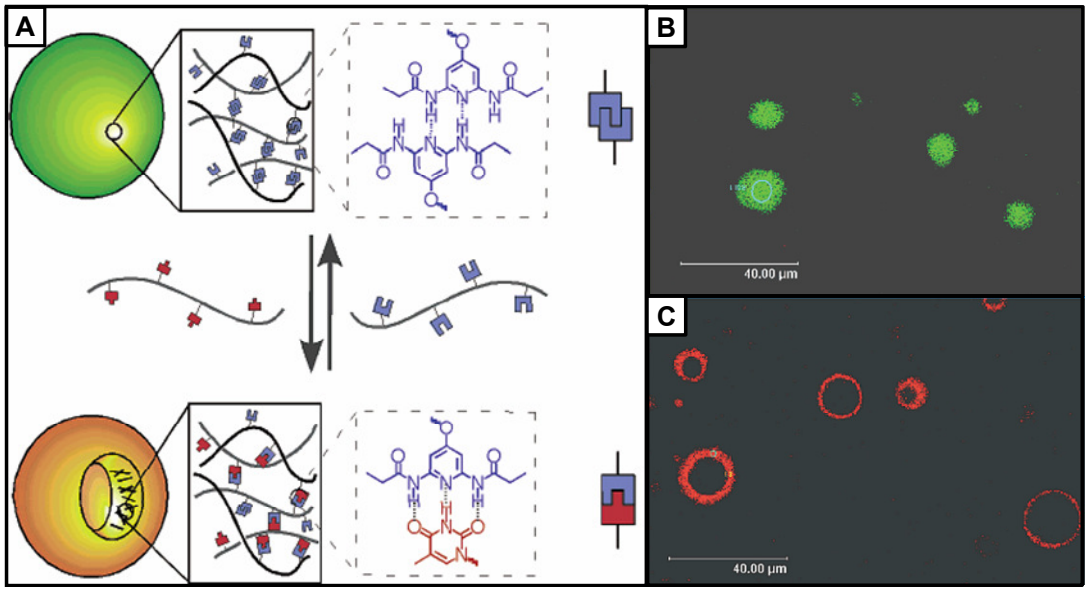


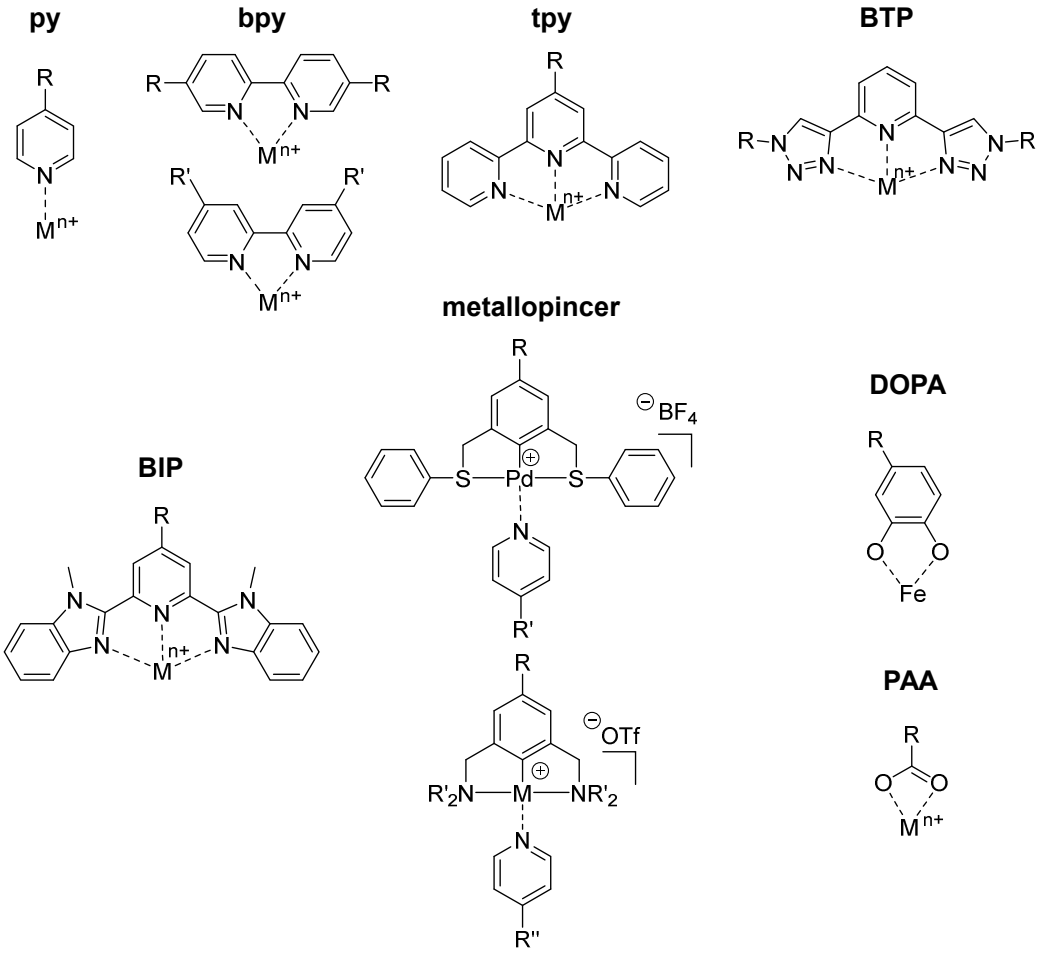

**Figure 9.** Transformation of supramolecular hydrogen-bonded polymer microspheres into vesicles. (A) Schematic illustration of the morphology change by specific hydrogen-bonding interactions. (B) Confocal fluorescence micrographs of labeled microspheres and (C) vesicles. Reprinted with permission from ref. 330. Copyright 2004 American Chemical Society.

Hydrogen-bonding polymer networks can even be prepared in ionic liquids, as reported by Noro et al.[334] In their work, an ABA triblock copolymer was synthesized containing endblocks that form

hydrogen bonds with a specifically designed homopolymer via interactions of pyridine and hydroxystyrene. The prime role of the ionic liquid is to assure good solvent conditions over a wide range of temperatures, allowing the network dynamics to be studied with significant variation of this parameter. FTIR measurements serve to quantify the number of hydrogen bonds per endblock as a function of temperature. This assessment shows that whereas the number of physical crosslinks is temperature-independent below the gelation temperature, the number of active hydrogen bonds within a particular crosslinking node strongly increases upon cooling.

#### 1.2.2.2 Metal Complexation

In addition to hydrogen bonding, metal complexation is another useful interaction to build up linear supramolecular polymers,[243] gels,[335] and networks.[336] For this purpose, a variety of different ligands have been developed, as summarized in Scheme 5. Among them, bi- (bpy) and terpyridine (tpy) ligands are particularly popular and have been studied extensively.[230,337] These ligands form chelate complexes with transition metal ions. Terpyridines coordinate to metal(II)-ions in a bivalent fashion, whereas bipyridines coordinate to metal(II) ions in a trivalent fashion. To exploit this, Schubert et al. prepared poly(methyl methacrylate) copolymers with terpyridine units in the side chains by free-radical polymerization of methyl methacrylate and a terpyridine-functionalized methacrylate.[338] Then, the terpyridine units were complexed by iron(II) and zinc(II) ions in mixtures of chloroform and methanol at low polymer concentrations (17–40 g $L^{-1}$), and the complexation was studied by UV–vis and viscosity measurements. Upon metal complexation, a characteristic UV–vis absorption band is observed at 558 nm, which is referred to the metal-to-terpyridine charge transfer, and an increase of the solution viscosity is found. Addition of zinc(II) results in lower viscosities than addition of iron(II), because zinc–terpyridine complexes are weaker than iron–terpyridine complexes. Further evidence for the weaker zinc binding is the observation that the zinc-complexed material can be redissolved after drying, whereas the iron-complexed polymer displays a gel-like appearance. To study the reversibility of the complex formation, the solutions were treated with HEEDTA (hydroxyethyl ethylenediaminetriacetic acid), which is a strong chelating ligand for transition metal ions and therefore acts as a competitive ligand to the terpyridine. Upon HEEDTA addition, the purple color of the iron(II) complexes disappears, and the viscosity of the solutions decreases significantly, demonstrating the reversible properties of these materials. In a similar approach, Tew and Calzia prepared terpyridine-modified poly(methyl methacrylate)s and investigated their complexation with copper ions.[339]




**Scheme 5.** Popular metal-complexation motifs used for supramolecular polymer-network formation.

Post-polymerization functionalization has also been applied for the synthesis of terpyridine-modified polymers.[340] In a recent approach, Schubert et al. employed this method to prepare poly(pentafluorostyrene) with terpyridines in the side chains.[341] First, poly(pentafluorostyrene) was synthesized by nitroxide-mediated polymerization with a narrow polydispersity index of just 1.08. In a second step, this polymer was converted with amine-functionalized terpyridine under microwave heating, which selectively substitutes the *para*-fluorines. Addition of iron(II) sulfate to a solution of the terpyridine-functionalized polymer in a mixture of chloroform and methanol leads to gelation at a polymer concentration of 33 g $L^{-1}$. In another work, Schubert et al. prepared metal-crosslinked polymer networks from linear and tri-arm poly(ethylene glycol) precursors, both functionalized with terpyridine at their OH-termini.[342] Quantitative functionalization of these precursors was achieved by conversion of the hydroxy-functionalized PEG derivatives with 4-chloro-2,2':6',2''-terpyridine at basic conditions. However, quantitative crosslinking with iron(II) chloride was not observed in methanol solutions, neither at room temperature nor at elevated temperature, but only a small quantity of crosslinked material precipitated from the solution. This observation was attributed to a strong

tendency of the tri-arm PEG to form intramolecular complexes acting as a chain stopper rather than as a crosslinker.

The advantages of both covalent and physical polymer networks have been combined in a terpolymer of poly(butyl acrylate) bearing terpyridine and oxetane units.[343] Non-covalent crosslinking of the terpyridine moieties is initiated by complexation to iron(II) ions, whereas addition of the Lewis acid $AlCl_3$ initiates covalent crosslinking by polymerizing the oxetane rings. As a result, this approach provides multiple possibilities for two-step crosslinking procedures and has the potential for developing new materials such as smart coatings with self-healing properties.

Conjugated metallo-supramolecular polymer networks that display interesting optoelectronic properties have been reported by Weder and co-workers.[344] Their approach is based on poly(*p*-phenylene ethynylene)s (PPE) that contain bipyridines in the main chain. The authors performed complexation studies of these polymers with several different transition metals ($Cu^+$, $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$, or $Cd^{2+}$) and obtained three-dimensional polymer networks in mixtures of chloroform and acetonitrile, which feature bpyPPE-metal-bpyPPE crosslinks. The complexes of $Zn^{2+}$ and $Cd^{2+}$ with $d^{10}$ electrons are light emissive, whereas the complexes of $Cu^+$, $Co^{2+}$, and $Ni^{2+}$ form nonradiative metal-to-ligand charge-transfer complexes with the polymers. Hecht and Meudtner used a similar approach and incorporated metal binding sites into the polymer backbone rather than into the side chains.[345] Their polymer synthesis is based on a step-growth polymerization process using multiple efficient Cu-catalyzed 1,3-dipolar cycloaddition reactions of 2,6-diethynylpyridine and 3,5-diazidobenzoate monomers. The monomers contain oligo(ethylene glycol) side chains to provide solubility in both nonpolar and polar solvents. Addition of various transition metal ions such as $Zn^{2+}$, $Fe^{2+}$, and $Eu^{3+}$ to such polymer solutions in acetonitrile instantaneously leads to gelation by tridentate complexation of the 2,6-bis(1,2,3-triazol-4-yl)pyridine (BTP) units to the metal ions. Whereas 1:2 complexes are formed in the case of $Fe^{2+}$, 1:3 complexes are created in the case of $Eu^{3+}$ due to a its larger atom radius,[346] exhibiting binding constants in the order of $10^7$ $M^{-1}$. Recently, in a similar approach, Weng and co-workers synthesized a macromolecular ligand containing the BTP unit in the main chain by using polyurethane chemistry.[347] Gels were prepared from this macromolecule by addition of $Zn^{2+}$ and/or $Eu^{3+}$ ions in mixtures of chloroform/acetonitrile or chloroform/THF. The gels containing $Eu^{3+}$ are stronger and more elastic than the gels formed with $Zn^{2+}$, which is consistent with the different binding characteristics of both metals. When chloroform/methanol mixtures are used, however, no gels are obtained, despite the fact that a slight increase of the solution viscosity is observed. This observation is referred to the chelating ability of methanol, which prevents formation of stable or long-living metal–BTP complexes. The $Zn^{2+}$-containing gel exhibits blue fluorescence in chloroform/acetonitrile on account of the Zn–BTP complex emission. When $Zn^{2+}$ is replaced by $Eu^{3+}$, more of the Eu–BTP emission shows up, resulting in a change of the gel's emission from blue to

characteristic red-orange. Both, the $Eu^{3+}$- and $Zn^{2+}$-containing gels are thermo-responsive and also sensitive to different chemical stimuli such as chelating agents. Moreover, both gel types show excellent self-healing properties: when freshly cut blocks of the gels are jointed together and kept in contact for several hours at room temperature, the newly formed gel bar is strong enough to sustain squeezing, bending, and stretching.

Rowan and co-workers took advantage of the ability of lanthanide metals to form complexes with up to three tridentate ligands and prepared multi-responsive metallo-supramolecular polymer gels from linear ditopic macromonomers.[348] For this purpose, a 2,6-bis(1-methylbenzimidazolyl)pyridine (BIP) moiety was attached to either end of an oligo-PEG core, and either lanthanum(III) and cobalt(II) or europium(III) and zinc(II) were used to form gels in a mixture of chloroform and acetonitrile. The $Co^{2+}$ or $Zn^{2+}$ ions act as linear chain extension binding units, whereas the $La^{3+}$ or $Eu^{3+}$ ions act as crosslinking components, as illustrated in Figure 10. By this means, four different gels were prepared containing Co/La, Zn/La, Co/Eu, and Zn/Eu. Upon removal of the solvent mixture, all four gels could be re-swollen in pure acetonitrile, in which they show thermo-responsive behavior: heating the Co/La gel to 100 °C results in a reversible gel–sol transition. At higher temperatures, the orange color of the Co-materials persists in solution, suggesting that it is the La–ligand interaction that is thermally broken. Addition of formic acid to the gels results in a loss of their mechanical stability due to strong binding of lanthanides to carboxylic acids. Furthermore, these gels were found to exhibit thixotropic (shear-thinning) behavior. Shaking the Zn/La gel, for example, results in a free-flowing liquid that can be reconverted into a gel state upon rest. This network reconstruction was investigated extensively by oscillatory shear rheology,[349] revealing that partial reconstitution of the network takes place instantaneously within 16 s, but complete regeneration of the formerly existing gel requires 18 min. It is hypothesized that reassembly appears to be a complex three step-process.

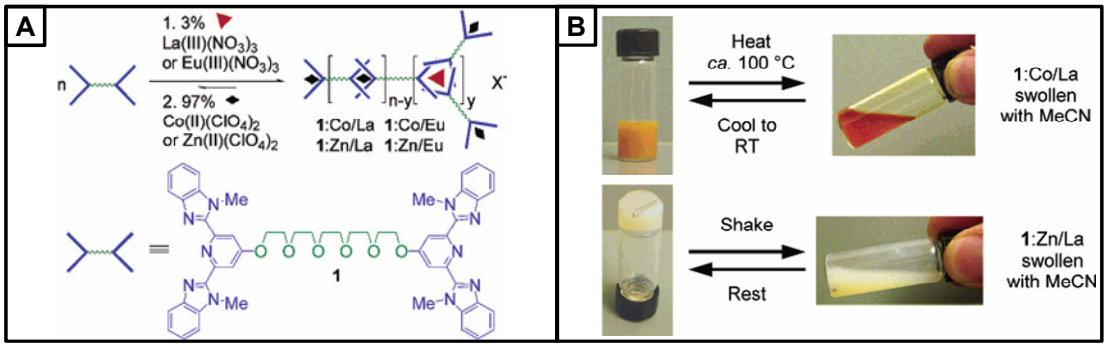



**Figure 10.** Metallo-supramolecular polymer gels as introduced by Rowan and co-workers. (A) Organogels formed by complexation of lanthanide and transition metal ions to 2,6-bis(1-methylbenzimidazolyl)pyridine (BIP) functionalized poly(ethylene glycol). (B) Thermo- and mechano-responsiveness of these gels. Reprinted with permission from ref. 348. Copyright 2003 American Chemical Society.

Rowan and Beck extended their work on these gel materials and showed that the stimuli-responsiveness depends on the type of metal and counter ion as well as on the amount of the swelling agent.[350] To deeply explore the properties of these gels and the nature of the gelation mechanism, Rowan et al. performed a series of experimental studies, including optical and confocal microscopy, dynamic light scattering, wide-angle X-ray diffraction, and rheology.[351,352] Morphological observation and X-ray diffraction suggest that the gelation occurs via flocculation of semicrystalline colloidal particles, which results in pronounced yielding and thixotropic mechanics of the gels. To investigate the influence of the solvent on the formation of BIP-based hydrogels, Rowan et al. used a mixed solvent system, consisting of a good solvent (dimethyl sulfoxide (DMSO)) with either a nonsolvent (water) or a poor solvent (ethylene glycol).[353] For each solvent system a composition window is located in which gels are formed. However, the gels drastically vary in turbidity from highly opaque in the water- or ethylene glycol-rich solvent mixture to highly transparent in the DMSO-rich mixture. Morphological and dynamic light scattering observations reveal that also in these materials, gelation occurs by the flocculation of semicrystalline colloidal particles: increase of the DMSO content leads to a reduction in the particle size, accompanied by an increase in sol concentration and in gel transparency along with an increase in the shear storage modulus. Scattering analysis indicates that the degree of crystallinity of the colloidal particles is dramatically decreased compared to those formed in pure acetonitrile; however, a new lamellar organization develops when the DMSO content increases. While for the previously described approaches, Rowan et al. used a pentaethylene oxide core equipped with BIP moieties on either end, in 2009 the same authors explored how little changes in the length of the ethylene oxide core influences the properties of the corresponding gels.[354] For this purpose, precursors with a tetra- and hexaethylene oxide core were prepared. Investigations of the gelation kinetics indicate that the rate of gelation decreases with the core length. In rheology under equivalent conditions of solvent quality and polymer concentration, the shear modulus of the pentaethylene core gels is substantially higher than that of the other core lengths. This finding is attributed to a lower solubility of the tetraethylene precursors resulting in smaller, more dense colloidal particles, and hence, a lower particle volume fraction. In case of the hexaethylene cores, the lower modulus is referred to their higher solubility, resulting in a larger sol fraction.

In analogy to their synthesis of hydrogen-bonding side-chain functionalized polynorbornenes, Weck et al. prepared the same polymers containing both metal-coordination sites and hydrogen-bonding motifs.[355] For the metal coordination, palladated metallopincer complexes that can coordinate to pyridine (py) moieties were used, whereas cyanuric acid or diaminopyridine moieties were employed for the formation of hydrogen bonds. By this means, polymer crosslinking is achievable in an orthogonal manner via metal coordination and hydrogen bonding upon addition of suitable small molecule crosslinking agents to the polymer solutions in chloroform. While metal-

complexation crosslinking results in a dramatic increase of the solution viscosities, crosslinking by hydrogen bonding only leads to minor changes of the viscosity. When just one type of supramolecular interactions is used for the polymer crosslinking, the second recognition motif along the polymeric backbone can serve to add further reversible functionalization of the polymer network. In a follow-up study, Weck et al. prepared similarly functionalized polynorbornenes containing both hydrogen-bonding and metal-coordination sites and studied the orthogonal decrosslinking of the resulting networks in 1-chloronaphthalene.[356] The hydrogen-bonded polymer networks are thermally reversible, whereas the metal-coordinated crosslinked networks mainly show chemo-responsive behavior. As a result, the metal coordination can be reversed by addition of suitable ligand displacement agents without affecting the hydrogen-bonded crosslinks, whereas in contrast, the hydrogen bonds can be selectively disassembled through competitive interactions with a monotopic end-capping agent without affecting the metal-coordinated crosslinks.

Craig and co-workers also used metallopincer complexes to form supramolecular polymer networks.[357,358] In their work, poly(4-vinylpyridine)s (PVP) were synthesized and subsequently crosslinked by addition of small molecule bifunctional palladium(II) or platinum(II) *N*,*C*,*N*-pincers in dimethyl sulfoxide. With this approach, the authors were able to control the dynamic mechanical properties of the gels, as discussed in detail in the Chapter “Dynamics in Supramolecular Polymer Networks”.

Although some examples of thermo-reversible metal coordination have been reported and discussed, very often metal complexation is too strong to be reversible within the temperature range accessible in a given experimental situation, most severely limited by the range of liquidity of the solvent or swelling agent. To extend this temperature window, Noro et al. demonstrated that ionic liquids are suitable as swelling medium for thermo-reversible polymer gels via metal–ligand coordination.[359] In their work, an ABA triblock was synthesized, the endblocks of which bore pyridine side groups which can coordinate to zinc(II) chloride. FT-IR spectroscopy reveals metal–ligand coordination in the ionic liquid, and temperature-ramp oscillatory shear measurements confirm thermo-reversible viscoelastic properties between a gel-like state and a liquid-like state.

As described previously, several studies of the melt rheological properties of supramolecular polymer networks assembled through hydrogen bonds have been carried out, but much less effort has first been spent on metallo-supramolecular polymers and networks. Recently, this gap was closed by Rowan et al., who prepared films of polytetrahydrofuran and poly(ethylene-*co*-butylene) functionalized with BIP moieties on both chain ends.[360] Zinc(II) ions were used to induce chain elongation, whereas europium(III) ions were used to induce crosslinking. When the amount of europium is increased, more thermo-responsive films of both polymer materials are obtained, although an increase of the concentration of $Eu^{3+}$ leads to a higher extent of supramolecular chain

branching. However, in the higher concentrated $Eu^{3+}$ materials, decomplexation of the weak $Eu^{3+}$ complexes is easy, resulting in facile supramolecular depolymerization. Furthermore, it is observed that the polymeric core plays a significant role in the material properties. The nonpolar poly(ethylene-*co*-butylene) core displays dramatic enhancement of the storage and loss moduli as well as the viscosity in comparison to the more polar poly(tetrahydrofuran) core.

### 1.2.3 Dynamics in Supramolecular Polymer Networks

The properties of supramolecular polymer networks display a delicate dependence on the equilibrium binding constant (thermodynamics) and the binding–unbinding dynamics (kinetics) of the constituent supramolecular crosslinking motifs, along with the accompanying polymer-physical characteristics.[239,361] To rationally control these properties, quantitative understanding of these relationships must be achieved. In this respect, it is necessary to control both the polymer and molecular dynamics independent of the thermodynamics of the supramolecular motifs. In a seminal work, this goal has been achieved by Craig and co-workers through the use of poly(4-vinylpyridine)s crosslinked with bifunctional metallopincers, as shown in Figure 11A.[357,358] Small variations in the steric hindrance between methyl and ethyl substituents on the pincers results in large changes of the complexation–decomplexation kinetics without significantly altering the binding–unbinding thermodynamics.[362] Vice versa, the binding thermodynamics can be varied by using two different metals, palladium and platinum, while retaining the same difference in the complexation–decomplexation kinetics between the methyl- and ethyl-substituted pincers.[357,358] With this approach, it turned out that it is not the crosslinking thermodynamics but the rate of crosslink dissociation that has a major impact on the dynamic material properties. This observation can be summarized as ‘slow dissociation means strong crosslinking.’ As a result, a master curve of the frequency-dependent viscosity is obtained when the viscosity is scaled by the ligand dissociation rate that was measured on low molecular weight model complexes, $k_d$, and when the frequency of the applied strain is scaled inversely by the same value, as shown in Figure 11B. Furthermore, a master curve of the storage modulus can be obtained if the frequency of the applied strain is scaled by the ligand dissociation rate, $k_d$, as shown in Figure 11C.

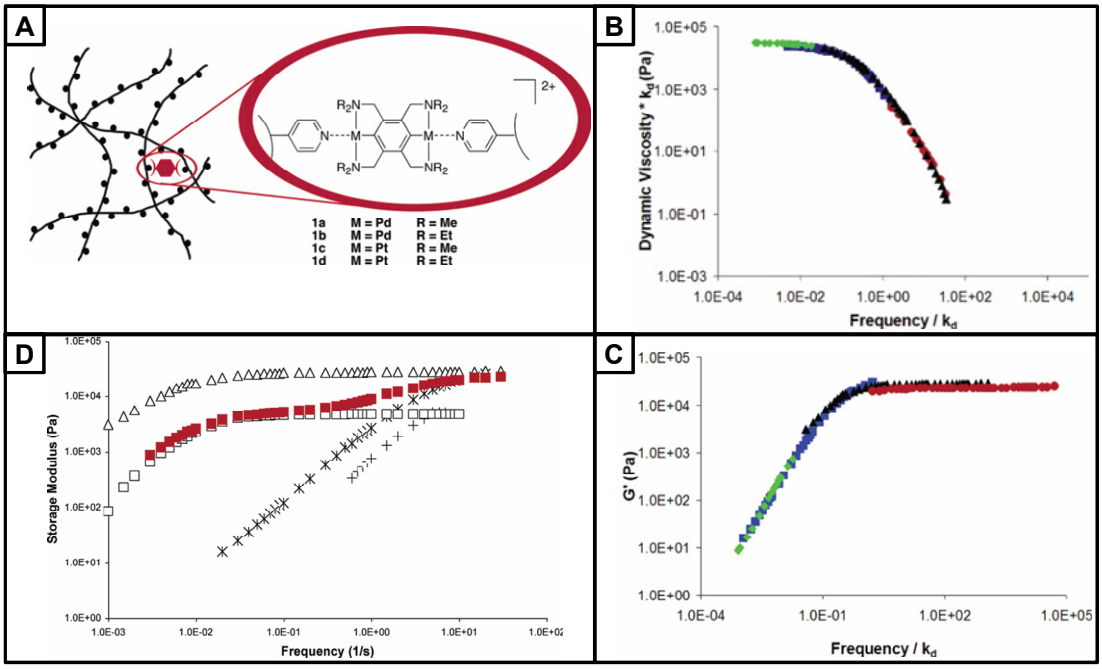



**Figure 11.** Independent control of the molecular dynamics and thermodynamics of metallopincer-crosslinked poly(4-vinylpyridine)s (PVP) supramolecular polymer networks in organic solvent. (A) Schematic of networks formed from PVP chains along with crosslinking bimetallic compounds (**1a–1d**). (B) Dynamic viscosity of the resulting supramolecular organogels scaled by the dissociation rate constant of the metal–pyridine crosslinking bond, $k_d$, versus the frequency of oscillation scaled by $k_d$ as well. (C) Storage modulus, *G'*, versus the frequency of oscillation scaled by $k_d$. (B),(C) Each of the networks consists of 5% (by metal functional group per pyridine residue) of (diamonds) **1a**, (squares) **1b**, (triangles) **1c**, and (circles) **1d** and PVP at 10% by total weight of network in DMSO at 20 °C. (D) Storage modulus *G'* versus frequency for (red full squares) 2.5% + 2.5% (**1b** + **1c**)–PVP, (open squares) 2.5% **1c**–PVP, (open triangles) 5% **1c**–PVP, (+) 2.5% **1b**–PVP, and (*) 5% **1b**–PVP at 10% by total weight of network in DMSO at 20 °C. Reprinted with permission from ref. 361, 363. Copyright 2005, 2007 American Chemical Society.

Experiments on supramolecular networks formed with multiple types of crosslinkers show that the response to an applied stress occurs through discrete contributions of each type of crosslinker rather than being an average of the contributing species.[363-365] Frequency-dependent measurements of such networks exhibit multiple plateau values in *G'*(ω), the corresponding inverse frequencies of which can be associated with the individual time constants of dissociation of the different crosslinks, as demonstrated in Figure 11D. Since the macroscopic dynamic response is controllable at the level of molecular associations, this effect has been called 'macromolecular analogue of the kinetic isotope effect'.[361]

The observations by Craig and co-workers have been supported by other groups who studied polymer networks based on different interactions, such as hydrogen bonding and host–guest complexation. For example, Meijer and co-workers found that the simple Maxwell model is applicable to describe the viscoelastic behavior of hydrogen-bonded UPy-functionalized polymer networks.[296] The resulting single relaxation time agreed well with the lifetime of the UPy dimer as measured independently by NMR spectroscopy. In addition, Scherman and co-workers applied the

Maxwell model to host–guest crosslinked cucurbit[8]uril (CB[8]) systems (see Chapter "Macrocyclic Inclusion Complexation" for details) and were able to determine the CB[8] ternary complex kinetics.[366] Moreover, Anthamatten demonstrated that systems consisting of both covalent and supramolecular crosslinks exhibit a discrete contribution from the dynamics of the supramolecular crosslinking.[259] To model both types of crosslinks, the authors developed a mechanical model that consists of a parallel array of an elastic spring (covalent contribution) and a Maxwell element (supramolecular contribution). They studied the creep, stress relaxation, and strain recovery and found that it is the hydrogen bond dissociation that dominates the elastomeric creep and shape recovery. Recently, the same group investigated how the dynamic behavior of functional poly(*n*-butyl acrylate) melts and crosslinked networks is influenced by hydrogen-bonding side groups of different associative strength.[367] They observed that copolymers containing weak hydrogen-bonding side groups behave like unentangled melts and exhibit higher storage and loss moduli with increasing amounts of binding groups. In contrast, copolymers containing strong hydrogen-bonding groups behave like entanglement networks.

The influence of the ligand-exchange kinetics on the material properties of supramolecular polymer networks was investigated by Sijbesma et al.,[368] who prepared reversible coordination networks by complexing diphenylphosphinite telechelic poly(tetrahydrofuran) with rhodium(I) or iridium(I) ions in chloroform solvent. Ultrasonication of both gels causes liquefaction after 3 min; re-gelation occurs after 1 min in the case of the rhodium(I) gel, but after 1.5 h for the iridium(I) gel. NMR measurements on model complexes show that the large differences in gelation times are in agreement with the ligand-exchange kinetics of the rhodium(I) and iridium(I) complexes. It is assumed that sonication of the gels results in ligand exchange, which changes the network topology without changing the coordination chemistry. Hence, upon stopping the ultrasonication, the gel fraction increases at a rate that is determined by the exchange kinetics of the metal complex.

### 1.2.4 Supramolecular Hydrogels

Early research on supramolecular polymer networks and gels was mostly conducted in the melt state or in unpolar organic media. This was because most of the initial supramolecular binding motifs were not strong enough to form networks in polar organic solvents or in water. Applications in the biomedical area, however, require the use of aqueous media. As a result, research on stronger supramolecular binding motifs along with the development of supramolecular polymer hydrogels has become a lively field of research. In this field, many approaches attempt to mimic Nature, where strong directed supramolecular interactions serve to form sophisticated self-assembled structures.

### 1.2.4.1 Hydrogen Bonding

**Synthetic Polymers**

Approaches to prepare hydrogen-bonded supramolecular hydrogels based on synthetic polymers are rare due to the challenge of designing binding motifs with associative interactions strong enough to withstand competitive hydrogen bonding with water. Recently, Meijer and co-workers addressed this challenge and reported the formation of hydrogen-bonded supramolecular hydrogels formed from UPy-terminated linear PEG with additional hydrophobic linkers between the UPy moieties and the PEG backbone.[369] To prepare these hydrogels, the UPy-PEG precursors were dissolved in an isotonic water solution at 70 °C and then cooled down to room temperature. The presumed mechanism of hydrogel formation is summarized and visualized in Figure 12A. In dilute solution, the polymers aggregate to form isolated nanofibers (Figure 12B) due to the presence of an urea motif incorporated into the hydrophobic blocks, which is prone to undergo lateral hydrogen bonding.[310] With increasing polymer concentration, these nanofibers form a transient network (Figure 12C). After approximately 16–24 h, the mechanical strength of the gel increases due to formation of supramolecular interchain crosslinks. The equilibrium mechanical properties of these materials are tunable by variation of the ratio of hydrophilic and hydrophobic parts of the polymer chains. Erosion of the hydrogels has been studied by observation of the release of an incorporated rhodamine dye (Figure 12D) and a fluorescent protein using fluorescence microscopy. Hydrogels formed from short PEG chains (6 kDa) release the rhodamine much slower than hydrogels formed from long chains (35 kDa), whereas a reversed effect is found for the protein release. Furthermore, the release is much slower when the hydrogels were aged compared to their freshly prepared counterparts. This finding can be discussed in view of an older hypothesis by Meijer and others, assuming that supramolecular polymer networks show self-repairing of network defects and reassemble into mechanically stronger structures with time.[296,306,321]

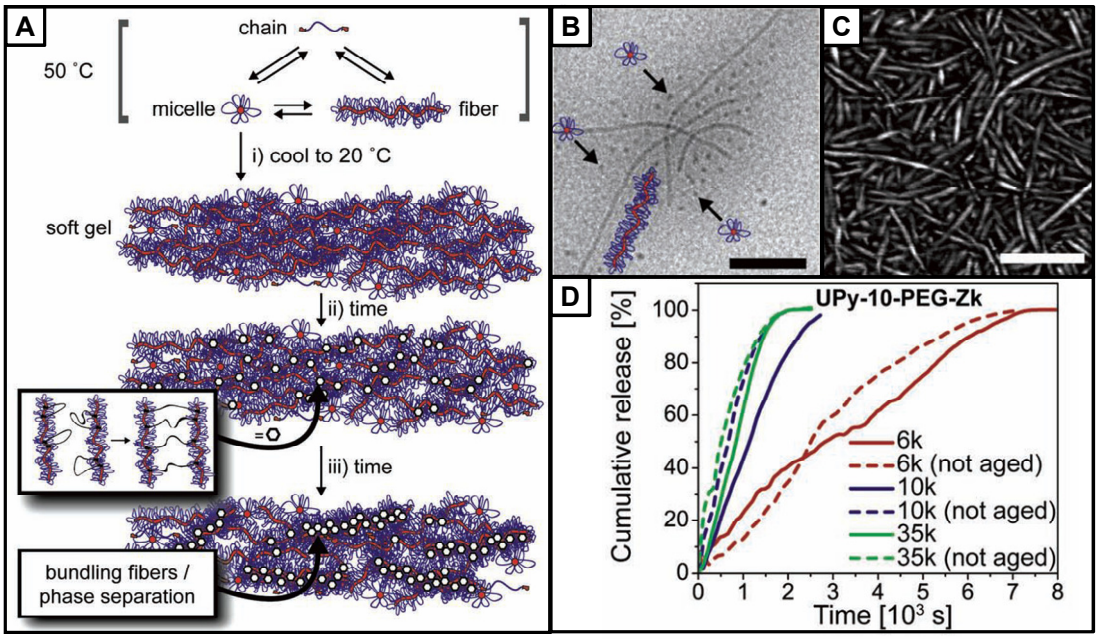



**Figure 12.** Formation and characterization of supramolecular hydrogels based on poly(ethylene glycol) chains that are end-functionalized with ureidopyrimidinone (UPy). (A) The process of hydrogel formation of UPy-modified PEG by hierarchical assembly of different structural units at 50 °C, comprising single chains, micelles, and fibers. (i) Upon cooling or increase of the polymer concentration, a soft hydrogel forms. (ii) Formation of supramolecular crosslinks after 16–24 h. (iii) Bundling of fibers, leading to phase-separating domains. (B) Cryo-transmission electron micrograph of fibers. The scale bar represents 100 nm. (C) Atomic force microscope phase image of the formed fibers (scale bar: 100 nm). (D) Release of rhodamine B from hydrogels prepared from PEG precursors of different molecular weights, as well as from freshly prepared and aged hydrogels. Reprinted with permission from ref. 369. Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA.

In a follow-up study, Dankers et al. extended the UPy-precursor toolkit to linear polymers containing UPy-moieties in the main chain.[370] The authors studied the rheological behavior of these materials, reported on their intrarenal behavior and tissue response, and proposed possible therapeutic applications. In another seminal work, Meijer et al. developed UPy-based dual-fiber networks as a synthetic analogue to fiber formation in the cytoskeleton.[371] For this purpose, mixtures of mono- and bifunctional polymers were used, which a priori form fibers in water; gelation could be achieved by decrease of the pH from 12 to 3.

Recently, Song and co-workers reported a very simple new method for fabricating tough hydrogels that are physically crosslinked by cooperative hydrogen bonding between a preexisting polymer and an *in situ* polymerized polymer.[372] These hydrogels were prepared by heating an aqueous acrylamide (AAm) solution in the presence of poly(*N*-vinylpyrrolidone) without any chemical initiators or covalent-crosslinking agents. Physical-chemical characterization, as well as molecular modeling indicates that the formation of strong cooperative hydrogen bonding between the preexisting poly(*N*-vinylpyrrolidone) and the *in situ* formed PAAm chains contributes to the gel formation. Mechanical tests of the as-prepared and swollen hydrogels demonstrated high tensile strengths, high tensile extensibility, and high compressive strengths at low moduli.

**Biopolymers**

In contrast to the just few examples of synthetic polymers that can be transiently crosslinked by hydrogen bonding, there is a huge variety of biopolymers that form supramolecular gels via hydrogen bonding. The most important representatives are polysaccharides such as cellulose,[373,374] starch,[375] agarose[376,377] and dextran,[378,379] the physical crosslinking of which occurs via hydrogen bonding of their hydroxy groups.

The preparation of hydrogels from native cellulose is a problem due to its extended hydrogen-bonded structure, which largely limits its solubility in both aqueous and organic media at ambient temperatures.[380] Recently, new solvents such as *N*-methylmorpholine-*N*-oxide or ionic liquids have been used to dissolve cellulose, thereby providing new opportunities to prepare hydrogels directly from the native polymer.[374] Another strategy to increase the solubility of cellulose in water is partial alkylation by etherification of the hydroxy groups to generate methyl, hydroxypropyl, hydroxypropylmethyl, or carboxymethyl cellulose.[380] Methyl cellulose forms hydrogels when its aqueous solutions are heated above a particular temperature,[381] most likely caused by hydrophobic interactions and exclusion of water between methoxylated regions of the polymer, along with hydrogen bonding between the remaining polymer hydroxy groups.

Because these cellulose derivatives are biocompatible, they have been tested in biomedical applications for the preparation of hydrogel matrixes.[374] However, these hydrogels turned out to degrade too rapidly for such applications; hence, blends of modified cellulose and other synthetic polymers or biopolymers including poly(vinyl alcohol)[382] or hyaluronic acid,[383] chitin,[384] chitosan,[385] or alginate[386] have been investigated. Zhang and co-workers prepared cellulose/poly(vinyl alcohol) hydrogels by using either chemical or physical crosslinking and compared the structure and properties of the different materials.[382] The chemical gels, which were prepared by crosslinking cellulose and PVA with epichlorohydrin, have a high swelling ratio but low mechanical strength. In contrast, the physical hydrogels, which were prepared by solution blending of cellulose and PVA and repeating freezing/thawing cycles, exhibit high mechanical strength due to a dense structure between cellulose and PVA. In another work, Shoichet and co-workers reported the development of a series of physical hydrogel blends composed of hyaluronic acid and methyl cellulose designed for independent delivery of one or more drugs.[387] The hydrogels exhibit several useful properties such as injectability, safe swelling, satisfactory diffusivity of molecules up to 150 kg $mol^{-1}$, high residual particle load, and significantly slower in-vitro degradation relative to earlier reports. The slow degradation rate of these hydrogels allows them to embed and release colloidal particles, rendering these composites useful for use in diffusion-limited and particle-mediated drug delivery from 1 to 28 days. In follow-up studies, the same authors investigated this hydrogel type as a scaffold for cell transplantation[388] and drug delivery,[389] focusing on the applicability of this system for treatment of

injuries in the spinal cord. The authors determined the effects of polymer concentration on the hydrogel mechanical strength, gelation time, and cell viability. They found the mechanical stiffness to be tunable via manipulation of methyl cellulose and hyaluronic acid content. By this means, the mechanical properties of the hydrogels could be optimized for the encapsulation of human umbilical tissue-derived cells with viabilities of up to 90% over a period of 3 days. As a result, these materials are promising vehicles for cell delivery and are presently being tested in ongoing in-vivo studies.

### 1.2.4.2 Metal Complexation

**Synthetic Polymers**

Despite the high strength of metal–ligand coordination and the huge number of ligands that have been developed to form synthetic supramolecular polymer gels based on metal-complexation crosslinking in organic solvents, there are only a few examples of corresponding hydrogels.

Fraser et al. demonstrated that bipyridine-centered and dimethacrylate-modified PEG macroligands form hydrogels by iron(II) complexation, which were further enforced by additional covalent crosslinking of the methacrylate moieties.[390] This approach served to form hydrogels with both supramolecular and covalent crosslinks that are stimuli responsive to acid, base, peroxides, and heat, all of which lead to ligand dissociation and metal-complex degradation, thereby entailing more loosely crosslinked materials.

Waite and co-workers mimicked the byssal threads of mussels, which allow them to physically attach to surfaces via the catechol-like amino acid dihydroxy-phenylalanine (DOPA),[391,392] and prepared hydrogels from catechol-functionalized synthetic polymers crosslinked with iron(III) ions.[393] Tris- and bis-catechol-iron(III) complexes possess some of the highest known stability constants of metal–ligand chelates ($K_{eq}$ up to $10^{40}$ $M^{-1}$).[394] The strength of complexation depends on the surrounding pH: whereas pH ≤ 5 leads to formation of weak monocatechol complexes, pH ≥ 8 promotes formation of bis- and tris-complexes. To achieve hydrogel preparation, the authors functionalized the hydroxy termini of tetra-arm PEG with DOPA moieties and mixed an aqueous solution of these precursor polymers with iron(III) ions at pH 5 to obtain a green-blue fluid. Upon increase of pH to 8, a sticky purple gel is formed. This physically crosslinked hydrogel displays almost the same elastic shear modulus as a comparable covalently crosslinked gel. However, after applying high strain, the chemical hydrogel is damaged irreversibly, whereas the physical hydrogel regains its elastic modulus within minutes. This is due to its reversible crosslinks, demonstrating the ability of these materials for self-healing. In a recent work, Waite et al. explored the utility of the DOPA derivative 3-hydroxy-4-pyridinone to form hydrogels with iron(III).[395] When this compound is coupled to tetra-arm PEG, hydrogels can be formed with iron(III) at physiological pH. Reversible gelation was

also achieved with other bio-relevant metal ions such as $Al^{3+}$, $Ga^{3+}$, and $Cu^{2+}$, which allows for tuning the gel dissolution profiles in controlled release applications.

Tong and co-workers reported the synthesis of a redox-responsive iron(III)-crosslinked poly(acrylic acid)-based (PAA) hydrogel.[396] Addition of iron(III) ions to a PAA solution produces a heterogeneous hydrogel due to fast binding of iron(III) to the carboxyl groups.[397] To prepare homogeneous gels, Tong et al. used citric acid to chelate the iron(III) ions and to slow down the binding rate. Then, the iron(III) ions were gradually released from the citrate-complex by decreasing the pH to form a homogeneous hydrogel. The gel can be decrosslinked by irradiation with sunlight for several minutes at room temperature through reduction of the iron(III) to iron(II). Vice versa, the hydrogel can also be reformed by exposure to oxygen, as shown in Figure 13.

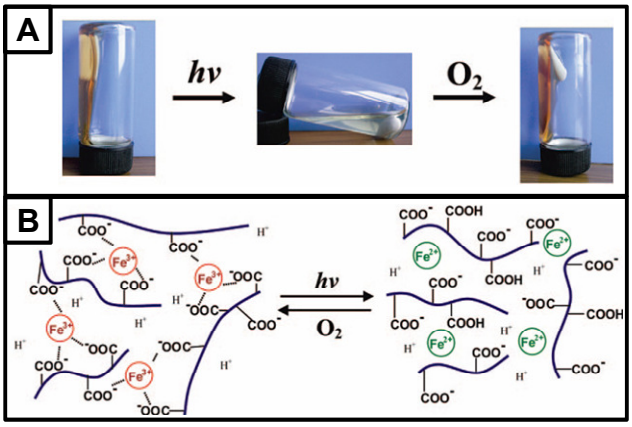



**Figure 13.** Reversible gel–sol–gel transition in an iron(III)-crosslinked poly(acrylic acid)-based hydrogel. (A) The homogeneous hydrogel disassembles by irradiation with simulated sunlight of 80 mW $cm^{-2}$ over a period of 12 min; subsequently, a homogeneous hydrogel can be reformed by exposure to oxygen in the dark for 5 days. (B) Schematic of the gel–sol transition. Reprinted with permission from ref. 396. Copyright 2008 American Chemical Society.

**Biopolymers**

The most commonly used biopolymer for the preparation of metal-crosslinked hydrogels is alginate. It is obtained from brown algae and consists of mannuronic and guluronic acids that are covalently linked together in different sequences or blocks.[398] The blocks are either similar or strictly alternating, and the exact composition depends on the origin of the alginate. Alginates can be prepared with a wide range of molecular weights, from 50 to 100,000 kDa, and can be crosslinked to form hydrogels by multivalent ions such as $Ca^{2+}$ or $Ba^{2+}$ through complexation to their carboxy groups. The mechanical properties of these gels depend on the ratio of guluronic and mannuronic acid in the polymer and on the concentration of the crosslinking cations.[399] Alginates are highly biocompatible and non-immunogenic[400] and are therefore widely applied in the pharmaceutical industry as excipients for drugs,[401] wound dressings,[402] and as synthetic extracellular matrixes for tissue engineering.[403] For these applications, the degradation of alginate-based hydrogels plays an

important role. Ionically crosslinked alginates dissolve at neutral pH upon loose of the divalent crosslinking cations, which results in uncontrolled and typically slow degradation kinetics in vivo.[404] To overcome this problem, Mooney et al. attempted to control the alginate degradation behavior by partially oxidizing the polymers and making them susceptible to hydrolysis.[405] The biocompatibility of these modified hydrogels was investigated by surficial culturing of myoblast cells, which adhered, proliferated, and differentiated at rate comparable to that of the same cells on unmodified gels.

Alginate hydrogels are also bio-inert, because mammalian cells do not have receptors to adhere to them. As a result, it is desirable to covalently modify alginate to promote cell attachment by coupling of short peptides,[403,406-408] fibronectin,[409] or collagen[410] to the alginate polymers.

### 1.2.4.3 Macrocyclic Inclusion Complexation

Inclusion complexation has developed to becoming another widely exploited supramolecular interaction for the formation of supramolecular polymer networks, most of them in water.[411,412] Several classes of macrocycles have been developed, including crown ethers,[413,414] porphyrins,[415,416] cyclophanes,[417] catenanes,[418] cavitands,[419,420] cryptophanes,[421] calix[*n*]arenes,[422] and carcerands.[423] Macrocyclic-based supramolecular gels can either be formed from low molecular weight precursors or from macromolecular building blocks. The following discussion focuses on the latter.

Among the different types of macrocycles, two classes of cavitands have gained particular attention in hydrogel chemistry:[411] cyclodextrins (CDs), which are the most extensively used macrocycles for forming supramolecular polymer gels,[424-427] and cucurbit[*n*]urils (CB[*n*]s),[428-430] which are a newer promising type of binding motif. These cavitands form inclusion complexes by locking a guest molecule within the cavity of the host. Binding in cyclodextrins occurs through hydrophobic interaction between the guest and the inner cavity; in water, strong binding ($K_{eq}$ = $10^5$ $M^{-1}$) occurs through additional solvophobic interaction.[431] Cucurbit[*n*]urils exhibit even higher binding constants of around $K_{eq}$ = $10^{15}$ $M^{-1}$ in water through additional ion–dipole interactions.[432] The exterior surface of cyclodextrins is hydrophilic due to the presence and high density of primary and secondary hydroxy groups; as a result, the commonly used α-, β-, and γ-CDs (six, seven, and eight D-glucose repeating units) are soluble in water; however the water-solubility of the β-CDs is relatively low. The solubility of CB[*n*]s varies in an odd–even fashion: whereas CB[5] and CB[7] are highly water soluble, CB[6] and CB[8] exhibit low water-solubility.[433] Both CB[*n*]s and CDs are nontoxic and biocompatible and therefore used in many life science applications and for the formation of supramolecular polymer networks. In 2000, cyclodextrins have been approved by the FDA to be used in drug delivery.[434]

CD-based hydrogels can be divided into several classes, as shown in Figure 14: A) Poly(pseudo)rotaxane hydrogels that contain CDs threaded to precursor-polymer chain ends or B)

to precursor-polymer chain branches. C) Hydrogels that contain guest groups in precursor-polymer side chains, crosslinked by addition of small-molecule CD dimers. D) Hydrogels obtained by mixing a polymer that is functionalized with CDs and another polymer that is functionalized with the corresponding guests. In the following, at least one example of each hydrogel class is presented.

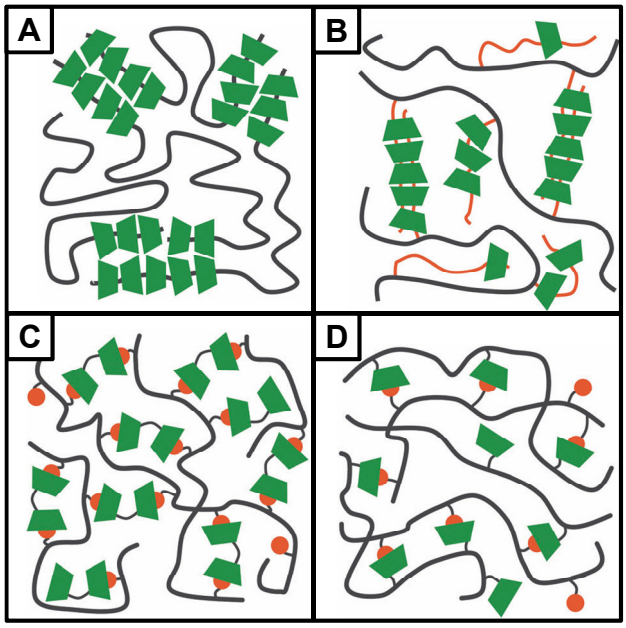



**Figure 14.** Schematic of different design principles for the formation of cyclodextrin (CD) based supramolecular polymer networks in water. (A) Crosslinking of hydrophilic polymer chains by threading CDs to either of their chain ends, thereby interconnecting them by hydrogen bonding between the exteriors of the CDs. (B) Similar crosslinking of polymer chains by threading CDs to dangling side-arm branches. (C) Crosslinking of precursor polymers that contain guest groups in their side chains by small-molecule CD dimers. (D) Crosslinking of precursor polymers that bear CD or guest moieties in their side chains. Reprinted with permission from ref. 411. Copyright 2012 Royal Society of Chemistry.

In 1994, Harada et al. reported the first example of hydrogels formed by the complexation of linear PEG chains with the inner cavity of α-CDs.[435] Hydrogen bonding between the exteriors of the bound cyclodextrins leads to the formation of crystalline domains and polymer-chain crosslinking. With this mechanism of crosslinking, several PEG-containing block copolymers have been developed and gelled by α-CDs.[436] The materials are biocompatible, thermo-responsive, and shear-thinning, which makes them ideal candidates for controlled release applications and drug delivery.[437,438] If γ-cyclodextrins are used, which have a larger ring diameter, very strong networks can be formed by threading two polymer chains through the macrocycle. By this means, Yui and co-workers prepared pH-responsive networks from γ-cyclodextrins and poly(ethylene glycol)-*block*-poly(ethylenimine).[439]

Adamantyl groups are known to be strongly intercalated within β-CD. Using this principle, Ritter and co-workers prepared adamantyl-containing copolymers of *N*-isopropylacrylamide and crosslinked them by a low molecular weight CD-dimer.[440] The networks exhibit a remarkable decrease in the lower critical solution temperature (LCST) of the copolymer, from 35 °C to around

15 °C, as a result of the restriction to the mobility and solubility of the polymer. In a recent approach, Ritter, Barner-Kowollik, and colleagues attempted to synthesize CD-based hydrogels by using linear double end-chain functionalized adamantyl poly(*N*,*N*-dimethylacrylamide) crosslinked by an CD-trimer.[441] In this approach, however, only viscous liquids and no gel-like materials have been obtained.

Instead of using CD-dimers for crosslinking, several research groups attached the CDs to a second polymer; in this strategy, mixing of the host- and guest-functionalized polymers can serve for hydrogel formation.[442,443] When the host and the guest molecules are attached to the same polymer, intrachain crosslinking is observed.[444] Several different synthetic and natural backbone polymers such as polyacrylic esters, poly(allylamine)s, polymethacrylates, polyesters, poly(ethylenimine)s, and chitosan, all equipped with CD side chains have been investigated in view of this approach.[445]

In addition to their inherent thermo-responsiveness, CD-based hydrogels that respond to pH, redox-potential, and light have been designed.[431,446-448] The first example of a light-responsive hydrogel obtained from a CD polymer and a guest polymer was reported by Harada and co-workers.[449] They used a glucan curdlan equipped with α-cyclodextrins and azobenzene-modified poly(acrylic acid). These materials are crosslinked by the complementary interaction of the α-CD unit and the *trans*-azo group. Upon UV-irradiation, the azo-moieties are isomerized to their *cis*-configuration, resulting in decomplexation and dissociation of the hydrogel network. This gel–sol transition is reversible, and polymer networks can be reformed by irradiation with visible light or heating to trigger re-isomerization of the azo groups to their *trans*-configuration.

Like CD-based hydrogels, also CB[*n*]-derived hydrogels can be divided into different classes: 1) Hydrogels that consist of a three-component system crosslinked by ternary CB[8] inclusion complexes and 2) hydrogels based on a two-component system of CB[6]@alkylammonium ion host–guest pairs. In the following, at least one example of each hydrogel class is given.

In 2010, Sherman and co-workers were the first who reported three-dimensional supramolecular crosslinked polymeric materials based on the CB[8] 1:1:1 ternary binding motif in water.[366] Copolymers were prepared that either contained pendant methyl viologen, which is a good first guest for CB[8], or naphthoxy derivatives, which are good second guests for CB[8], as visualized in Figure 15A. Addition of CB[8] to a colorless solution of the two copolymers leads to transformation into a highly viscous, colored supramolecular hydrogel with a crosslinking density that is controlled by the amount of CB[8]. The hydrogels exhibit solid-like mechanical properties at 5 wt% in water, with plateau moduli of 350–600 Pa at a crosslinking density in the range of 2.5–10%, which is complementary to other supramolecular hydrogels that exhibit higher mechanical strength.[450,451] In a following work, Sherman and co-workers reported on ultra-high water content hydrogels (up to 99.75% of water) based on the same host–guest complexation but derived from renewable cellulosic

derivatives.[452,453] The authors demonstrated shear-thinning behavior as well as sustained release of model proteins from these materials (Figure 15B) over the course of 160 days, which shows their potential for biological applications.

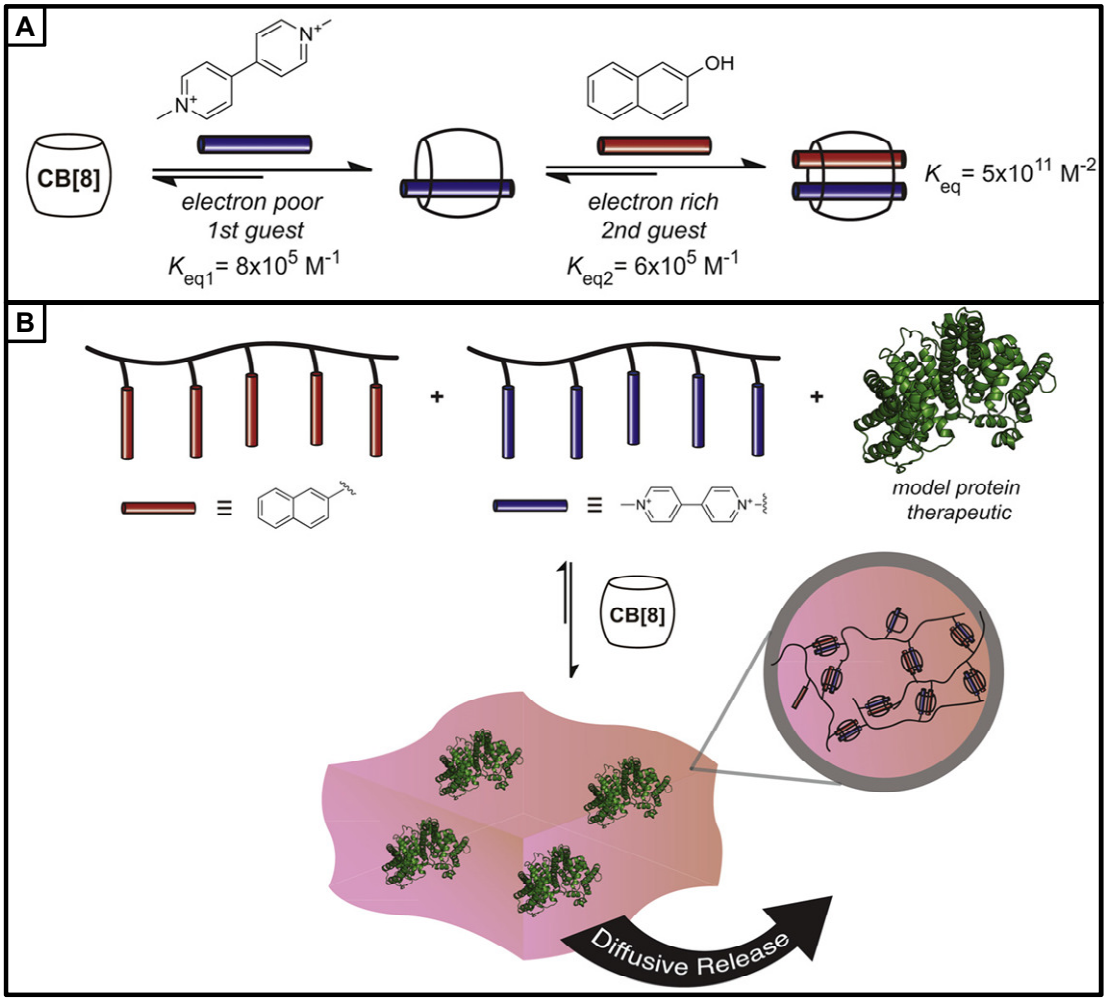



**Figure 15.** Hydrogel formation by two-step, three-component binding of cucurbit[8]uril (CB[8]). (A) Copolymers that contain pendant methyl viologen are good first guests for CB[8], whereas copolymers that contain naphthoxy derivatives are good second guests for CB[8]. (B) Schematic of the preparation of protein-laden high water-content CB[8]-hydrogels. Reprinted with permission from ref. 453. Copyright 2012 Elsevier.

In another work, Kim et al. demonstrated the supramolecular assembly of CB[6]uril-conjugated hyaluronic acid and diaminohexane-conjugated hyaluronic acid to biocompatible hydrogels in the presence of living cells.[454] The authors exploited the high binding affinity and selectivity of CB[6] toward alkylammonium ions in aqueous solution ($K_{eq}$ = $10^{10}$–$10^{12}$ $M^{-1}$). Excellent cell viabilities of more than 90% were observed after incubation of the cell-laden hydrogels for 3 days. Moreover, the hydrogels are degradable by enzymes, which is an important property of artificial extracellular matrixes. Favorably, the presence of free alkylammonium groups in the polymer network allows the attachment of functional tags such as dyes and adhesion peptides.

### 1.2.4.4 Ionic Interactions

Hydrogels based on ionic interactions are extremely robust materials and therefore provide an alternative to covalently crosslinked hydrogels.[455,456] This is because of the extremely strong binding of the multivalent, oppositely charged precursor polymers. Nevertheless, these materials are highly responsive to and degradable by changes of the pH or salt concentration in the swelling medium. Ionic hydrogels can be obtained from both synthetic precursor polymers and from biopolymers.

**Synthetic Polymers**

Polyelectrolytes based on block copolymers are widely used for the preparation of hydrogel materials. Often, an ionic midblock serves as the water soluble component, whereas neutral and hydrophobic endblocks lead to network formation by collapsing into local phase-separated domains.[457] In contrast, there are only few examples of hydrogels solely based on ionic interactions. This is because mixing of oppositely charged polyelectrolytes is often impossible, favoring macroscopic phase separation; this, however, can be prevented when copolyelectrolytes are used that contain neutral hydrophilic midblocks. In this case, phase separation is limited to occur just microscopically, leading to micelles that consist of a core containing a polyelectrolyte complex and a corona of neutral solvophilic blocks.

Hawker et al. used such an approach and prepared ionic ABA triblock copolymers that consist of a central PEG block and charged endblocks including varying numbers and types of ionic functional groups such as sulfonate, carboxylate, ammonium, and guanidinium, as shown in Figure 16A.[458] By synthesizing triblock copolyelectrolytes that are identical in all aspects except for the ionic groups, the dependence of the hydrogel formation on the effects of polyelectrolyte p*K*a could be probed, as shown in Figure 16B. When solutions of copolyelectrolytes bearing weak ionic groups like ammonium and carboxylate or ammonium and sulfonate are mixed, transparent viscous fluids are formed. In contrast, mechanically robust hydrogels are obtained through the strong interaction of carboxylate- and guanidinium-functional triblock copolyelectrolytes. When copolyelectrolytes bearing the strongest ionic groups, sulfonate and guanidinium, are mixed, the most stable, mechanically resilient gels are formed even at low polymeric concentrations (3–5 wt%). When incubated into sodium chloride solutions, these hydrogels exhibit enhanced responsiveness with increasing salt concentration. In a following work, Tirrell et al. investigated the effect of variations in polymer concentration, salt concentration, pH, and stoichiometry of the charged moieties on the structure and viscoelastic properties of these coacervate hydrogels.[459]

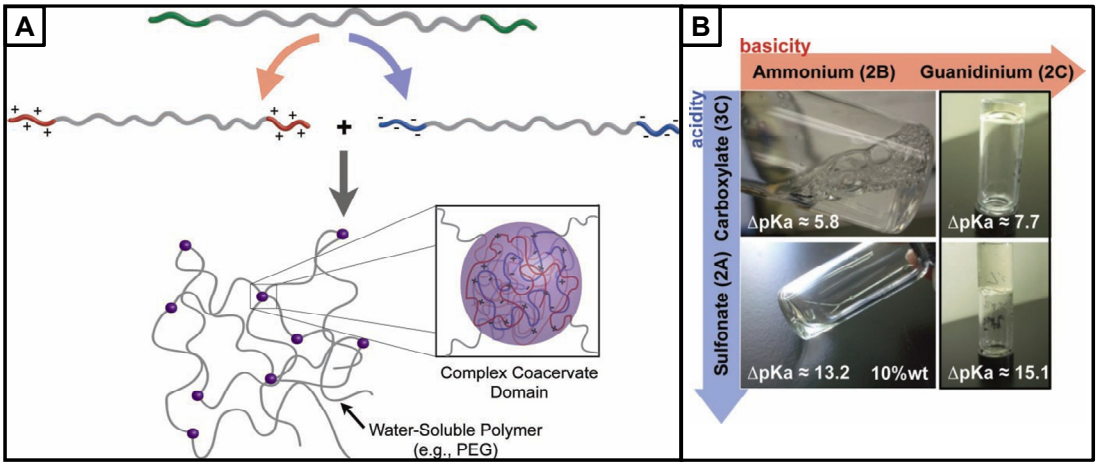



**Figure 16.** Coacervate-driven hydrogel formation. (A) Oppositely charged ionic ABA triblock copolyelectrolytes are obtained from a triblock copolymer precursor. Mixing dilute aqueous solutions of each results in coacervate crosslinking and formation of a network structure. (B) Effect of the polymer ionic strength on the hydrogel properties. Reprinted with permission from ref. 458. Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA.

In a similar work, Cohen Stuart et al. prepared ABA triblock copolymers with negatively charged endblocks and a water-soluble neutral midblock as well as a positively charged homopolymer.[251] When aqueous solutions of the oppositely charged polymers are mixed, a highly viscous and transparent gel is formed spontaneously. In agreement to the work of Hawker et al., small-angle X-ray scattering (SAXS) reveals that the gels consist of a network of interconnected polyelectrolyte-complex micelles wherein which the neutral middle blocks form bridges. The network properties are tunable by the concentration, temperature, ionic strength, pH, and charge composition.

In another approach, Aida and co-workers end-capped linear PEGs with dendrimers bearing positively charged guanidinium groups.[450] When these materials are mixed with negatively charged polyacrylates and clay nano sheets, hydrogels are obtained. The mechanism of hydrogel formation is explained as follows: When the clay nanosheets, which are highly entangled with one another, come into contact with polyacrylates, they are exfoliated and dispersed homogenously owing to the mutual repulsion caused by a possible site-specific wrapping of their positively charged edges with the anionic polyacrylates. The nanosheets, which subsequently contain highly negatively charged surfaces, are then crosslinked by the guanidinium-containing PEG dendrimers to form a 3D network in water. The hydrogels exhibit an exceptional mechanical strength (*G'* up to $10^6$ Pa) as well as rapid self-healing. In a follow-up study, the same group was able to prepare similar hydrogels by using linear ABA triblock copolyethers carrying guanidinium groups in their endblocks.[451] These hydrogels are as tough as the dendrimer-based ones, but the linear binders can be obtained by much less elaborate syntheses from starting materials that are readily available. Moreover, the gelling water can be freely replaced with ionic liquids and organic fluids, affording novel iono- and organogels.

**Biopolymers**

Chitosan is a well-investigated example of a polycationic biocopolymer composed of 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose. It is prepared industrially by hydrolysis of the aminoacetyl groups of chitin, which is a naturally available polymer.[460] Because chitosan is non-toxic, biocompatible, and biodegradable, it has attracted considerable interest in a wide range of biomedical and pharmaceutical applications,[461] including drug delivery,[462] cosmetics,[463] and tissue engineering.[460] Chitosan has primary hydroxyl and cationic amino groups that can react with a number of multivalent anions to form hydrogels.[460] Various polyelectrolyte hydrogels with natural polyanions including xanthan[464], hyaluronic acid,[465] alginate,[466] collagen,[467] pectin,[468] and gelatin[469] have been prepared.

Xanthan has a cellulosic backbone and a trisaccharide side chain consisting of D-glucose, D-mannose, and D-glucuronic acid. The first report on chitosan–xanthan hydrogels has been made by Dumitriu and co-workers in 1994.[464] Stable hydrogels exhibiting a fibrillar structure, as observed by electron microscopy, were formed that contain very high amounts of water (up to 95%). Dumitriu investigated the structure–property relationship of these materials by variation of the precursor-polymer composition and found the mechanism of gelation to be based on coacervation.[470] Furthermore, electron microscopy reveals the formation of fibrillar structures, which makes this system interesting for biomedical applications such as those in drug delivery and cell encapsulation. As a result, numerous publications and patents have been released dealing with the applicability of these materials from immobilization of enzymes to dermatology.[471,472]

### 1.2.4.5 Hydrophobic Interactions

Hydrophobic interactions play an important role in the formation of large biological systems, but they can also be used to generate synthetic hydrogels.[473] For this purpose, hydrophobic sequences are incorporated within hydrophilic polymer-network chains. To achieve this, micellar polymerization is a commonly used technique:[245] a water insoluble hydrophobic comonomer is solubilized within the micelles and is then copolymerized with a hydrophilic monomer in aqueous solution by free-radical addition polymerization. By this means, *n*-alkylacrylamides or *n*-alkyl methacrylates with alkyl chain lengths between 4 and 12 carbon atoms could be copolymerized with acrylamide to obtain tough hydrogels.[474,475] Copolymerization of acrylamide with dodecyl methacrylate (C12) yielded hydrogels exhibiting elastic moduli of around 1 kPa.[475] The hydrophobic associations acting as temporary crosslinks are strong enough to be retained in water during swelling of the gel network. Static light scattering reveals that these hydrogels are more homogeneous than the corresponding gels prepared by a chemical crosslinker, which is attributable to the mobility of the crosslinking nodes. Large hydrophobic monomers such as stearyl methacrylate (C18) and dococyl acrylate (C22) with

very low water solubility could also be copolymerized with acrylamide in a micellar solution by the addition of sodium chloride.[245,476,477] The salt leads to micellar growth and solubilization of the hydrophobes. The hydrogels thus obtained exhibit excellent self-healing properties. When fractured, they can be repaired by jointing their fractured surfaces to self-heal at room temperature, after which the materials regain their original mechanical properties. However, when swollen in water, such hydrogels lose their capability of self-healing due to the extraction of SDS micelles from the gel network by dilution and washing procedures. Recently, Okay and co-workers reported that complete healing of these swollen physical gels is achievable by treatment of the damaged area with an aqueous solution of wormlike SDS micelles. [478]

As an alternative to alkyl side chains, fluorocarbon hydrophobes can also be used to prepare hydrogels based on hydrophobic interactions.[479-481] In water, fluorocarbon motifs display even stronger hydrophobic association than hydrogenated hydrophobes, and fluorocarbon groups possess excellent chemical and biological inertness, along with extra favorable high gas permeability, all of which making them useful for biomedical applications.

### 1.2.5 Applications

#### 1.2.5.1 Self-Healing

Micro-crack formation and crack propagation are a common cause of material failure. To overcome this problem, supramolecular polymer networks have been developed that can self-heal based on the dynamic nature of their reversible crosslinks.[482] The healing can either occur in an autonomous fashion or upon exposure to an external stimulus such as heat, light, pressure, or mechanical stress.

In a much-noticed approach, Leibler et al. prepared self-healing supramolecular polymer networks based on multiple hydrogen bonding.[257] For this purpose, commercially available fatty acids with various degrees of branching were functionalized with hydrogen-bonding ureas that are capable to self-assemble, thereby forming glassy plastic materials ($T_g$ = 28 °C). These materials were transformed into elastomers by swelling in dodecane ($T_g$ = 8 °C), exhibiting an elongation to break of 600%. If ruptured, the remaining fragments are capable of self-healing within a few minutes by hand-pressing them together. Rheological studies on the repaired rubber confirmed restoration of its original mechanical properties.

In 2011, Weder and co-workers reported the first healable metallo-supramolecular polymer networks.[483] They used telechelic macromonomers based on an amorphous poly(ethylene-*co*-butylene) core with 2,6-bis(1-methylbenzimidazolyl)pyridine (BIP) ligands and crosslinked the polymers by transition or lanthanide metal salts. Mechanical defects are healable by exposure of the crack to UV light: the metal–ligand motifs are electronically excited and the absorbed energy is

converted into heat, which causes temporary disengagement of the metal–ligand motifs and thereby entails quick and efficient defect healing.

Recently, Schubert et al. investigated the healing properties of terpyridine-based films.[484,485] Methacrylate copolymers containing terpyridine moieties in the side chain were prepared, and iron(II) and cadmium(II) salts were used for crosslinking. In the case of iron(II) crosslinking, superfacial scratches could be healed upon modest thermal treatment at 100 °C. The use of $Cd^{2+}$ as a crosslinker even reveals improved self-healing properties such as lower healing temperatures (<75 °C) and faster healing.

Colquhoun, Hayes, and co-workers demonstrated that supramolecular polymer blends crosslinked by electronically complementary aromatic π-systems exhibit self-healing properties.[486,487] In this approach, low molecular weight polyimides that contain multiple π-electron receptor sites bind to complementary π-electron-rich pyrene termini of telechelic polysiloxanes. The obtained materials exhibit relatively poor mechanical stability, but they are healable at temperatures above 80 °C. The mechanism proposed for this thermo-reversible healing involves disruption of the intermolecular π–π stacking crosslinks, followed by flow and reassociation of disrupted polymer chains. To improve the mechanical properties, a second generation of materials was developed by modifying the polymeric backbone while maintaining the π-systems.[247] These materials exhibit improved healing properties, because healing is not only possible directly after fracture, but also if fragments are separated for more than 24 hours and then brought into contact again. Recently, the same authors developed healable materials containing two distinct types of supramolecular interaction, hydrogen bonding and π–π interactions.[248]

#### 1.2.5.2 Shape Memory

When covalent and supramolecular crosslinking is combined, shape-memory materials can be obtained. If these materials are heated to a temperature where the supramolecular crosslinks break, only the remaining permanent crosslinks give rise to an elastic response upon external deformation. If such deformation is applied along with subsequent decrease of the temperature, reassociation of the supramolecular crosslinks locks-in the deformation. If the temperature is increased again, the supramolecular bonds are re-broken, entailing unleash of the stored elastic response of the permanent crosslinks, thereby restoring the original shape of the material.

To prepare films exhibiting multi-responsive shape-memory properties, Kumpfer and Rowan used a covalent photo-crosslinking process that allows the formation of complex permanent shapes and a metal–ligand hard phase serving as the reversible phase to fix an independent temporary shape.[488] For this purpose, low molecular weight polybutadiene was end-capped with BIP ligands that form high molecular weight metallo-supramolecular polymers upon addition of metal salts. In addition,

the polymer solution contained a tetra-functional thiol along with a photoinitiator. Mechanically stable films are obtained by solution casting, comprising supramolecular metal–ligand crosslinks but so far no covalent crosslinks. Hence, the films can be brought into a variety of shapes, and exposure to UV-light induces thiol–ene crosslinking, thereby fixing this shape. If the temporary metal–ligand phase is disrupted by a stimulus such as light, heat, or chemicals, an independent temporary shape can be created. Removal of the stimulus while the material is deformed allows the metal–ligand complexes to reform and to lock-in the new shape. Applying another stimulus induces recovery back to the permanent shape, as illustrated in Figure 17.

Besides metal complexation, also hydrogen bonding can be applied to fix a temporary shape and to create shape-memory materials.[489-491]

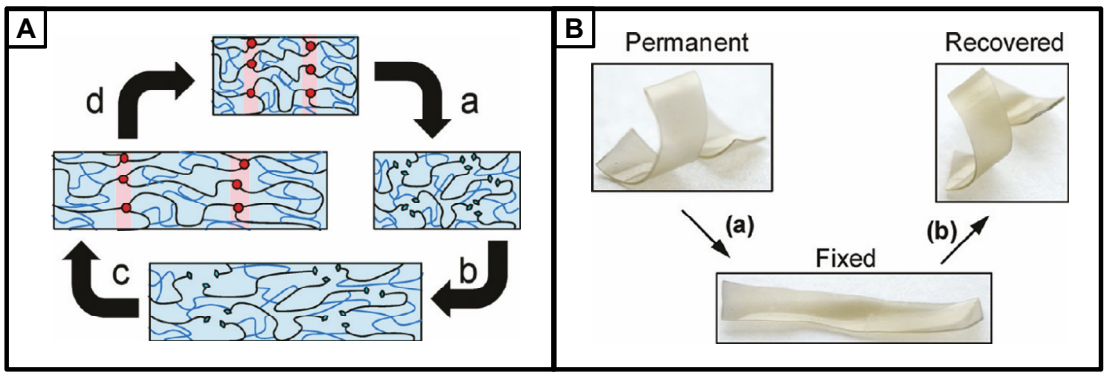



**Figure 17.** Access to shape-memory materials from photo-crosslinked metallo-supramolecular polymers. (A) Schematic of the formation of shape-memory materials using light as a stimulus: (a) UV light is absorbed by the metal–ligand complexes and is converted to localized heat, which disrupts the metal complexation; (b) the material can then be deformed; (c) removal of the light while the material is deformed allows the metal–ligand complexes to reform and to lock-in the temporary shape; (d) additional exposure to UV light allows a return to the permanent shape. Reprinted with permission from ref. 488. Copyright 2011 American Chemical Society.

### 1.2.5.3 Drug Delivery

Because of their reversibility and stimuli-responsiveness, supramolecular polymer gels have gained attention for the encapsulation of drugs and their subsequent controlled release at a targeted site.[389,492,493] The release can occur through auto-degradation of the hydrogels by dilution of the surrounding medium, through specific stimuli at the target site, for example, in response to a decreased pH in tumor tissue,[494] or through external stimuli.

Catheter delivery of drugs to the heart is a great challenge, because after injection, the drugs are immediately pumped out of the heart again. To overcome this problem, a carrier matrix is needed that is injectable through a long catheter in a solution state but then instantaneously gels. For this purpose, Dankers and Chamuleau et al. developed a UPy-based hydrogel that can be used as a carrier for catheter-injection of drugs into infarcted myocardium.[495] They prepared a supramolecular

hydrogel that can be switched into a liquid at pH > 8.5 with a viscosity low enough to enable passage through a catheter. Then, the natural pH of the tissue instantaneously transforms the injected solution into a drug-loaded hydrogel reservoir. Local in-vivo delivery of contrast agents and growth factors by hydrogel auto-degradation was demonstrated in a large animal model of ischemic heart disease.

Zhang and co-workers reported supramolecular hydrogels for dual drug release prepared by inclusion complexation.[496] Heparin was first conjugated to poly(ethylene glycol) methyl ether and then used to form a gel with α-cyclodextrin in aqueous solution. In addition, bovine serum albumin was encapsulated as a model protein drug. The resulting hydrogels show dual release behavior for the encapsulated model protein drug and conjugated heparin, along with good anticoagulant and blood-compatible properties.

Weber and co-workers developed a hydrogel for time-scheduled vaccination that consists of biohybrid materials crosslinked via multiple supramolecular interactions.[497] The hydrogel is based on two 8-arm poly(ethylene glycol) species with end groups that are either functionalized with fluorescein or with a humanized single-chain antibody fragment (scFv) specifically binding fluorescein. Binding of the scFv to fluorescein crosslinks the polymers to forming a gel. The hydrogels were implanted subcutaneously into mice and could be dissolved upon oral administration of fluorescein. By this means, vaccines and pharmaceuticals can be released from the hydrogel in a remote-controlled manner.

#### 1.2.5.4 Microgels for Cell Encapsulation

If microgels are prepared through the use of reversible physical interactions, they are degradable or can be cleaved on demand, rendering them attractive for the reversible encapsulation of additives, including cells. For this purpose, several groups developed degradable microparticles on the basis of reversible crosslinking of natural polymers such as alginate,[498] agarose,[499,500] or gelatin,[501] all of which serving for cell encapsulation.

Weitz et al. reported a microfluidic technique to encapsulate living yeast cells in alginate hydrogel microparticles generated from monodisperse double-emulsion templates.[502] First, double emulsions were prepared that consist of an alginate drop surrounded by a mineral oil shell, as shown in Figure 18A. Once the alginate drop separates from the mineral oil shell and comes into contact with $Ca^{2+}$ ions in the continuous phase, droplet gelation occurs (Figure 18B). The viability of cells encapsulated into the resulting alginate microparticles was determined to be 65% after one week.

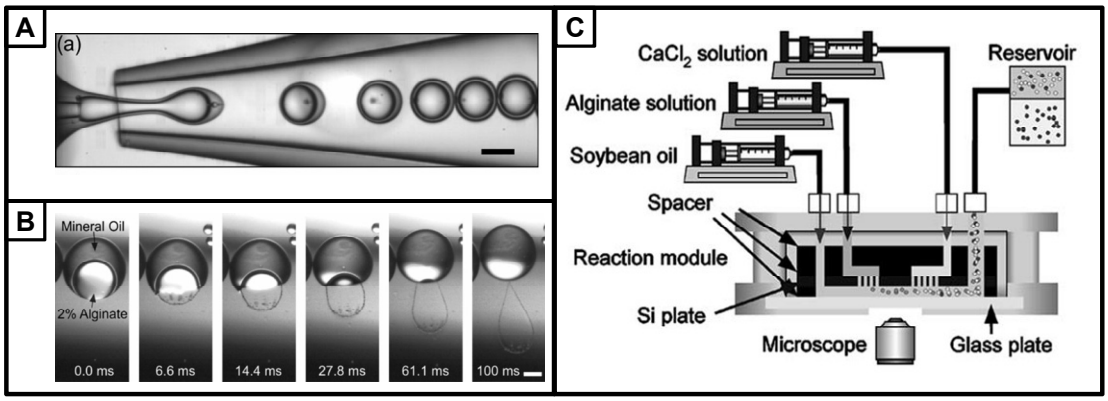



**Figure 18.** Preparation of cell-laden alginate microgel particles. (A) Optical image of alginate/mineral oil double-emulsion drop formation. (B) Optical images showing the separation of the inner alginate drop from the mineral oil shell. Both scale bars denote 100 µm. (C) Schematic of the experimental setup to prepare alginate microgel particles using a micro-nozzle array. Reprinted with permission from ref. 502, 503. Copyright 2005, 2012 Elsevier and WILEY-VCH Verlag GmbH & Co. KGaA.

In a related work, Nakajima and co-workers formed calcium alginate beads complexed with human kidney cells using a micro-nozzle array, as shown in Figure 18C.[503] In this approach, an aqueous alginate solution containing the cells was extruded through a micro-nozzle and flow-focused with an oil phase to form alginate droplets. These alginate droplets were immediately reacted with $CaCl_2$ droplets downstream of the oil flow by collision and subsequent droplet coalescence, thereby forming calcium alginate gel beads with sizes between 50 and 200 µm depending on the flow rates. The viability of encapsulated cells was estimated to be around 70%.

By using synthetic poly(*N*-isopropylacrylamide)-*block*-polystyrene precursor polymers in combination with droplet microfluidics, Kumacheva et al. were able to generate physically crosslinked nanofibrillar microgels.[504] The microgels can be formed under physiological conditions and rapidly dissociated upon cooling to 25–27 °C. This makes them interesting as scaffolds for temporary cell encapsulation and subsequent cell release for further cell characterization.

# 2 Scientific Goals

The development of supramolecular polymer networks with rational design requires a deep and fundamental understanding of the physical and chemical properties of these materials. A particular view should be addressed to the complex interplay between the structure, dynamics, and mechanics. To derive this knowledge, existing material platforms have several major limitations: First, most of them focus on just one type of supramolecular interaction only, either hydrogen bonding or metal complexation. Second, many existing supramolecular network systems do not allow for a broad variation of the type and polarity of solvent, thereby obstructing the kinetics and thermodynamics of network formation and rearrangement to be tuned in this way. Third, the crosslinkable precursor polymers are typically formed by copolymerizing a main monomer along with modified supramolecular crosslinkable derivatives in a one-pot polymerization. As a result, the precursor polymers vary from batch to batch in terms of their degrees of side-chain functionalization, molecular weights, and substitution patterns.

All these limitations prevent consistent and comprehensive investigation and comparison of different types of supramolecular polymer networks and gels to derive general structure–property relations. This thesis aims to overcome this shortcoming. For this purpose, a modular construction kit was developed based on just *one* common backbone polymer that can be transiently crosslinked through hydrogen bonding and metal complexation in different solvents, without alteration of the backbone polymer material. By this means, the impact of the type and strength of interchain crosslinking on the network mechanics and dynamics can be studied with high consistency.

Besides the strength of transient chain crosslinking, the mechanical properties of supramolecular polymer gels are mainly determined by the polymer network topology. Polymer networks often exhibit polydisperse mesh sizes, along with structural imperfections such as loops and dangling chains. To facilitate consistent investigation and rational design, the effects of polymer crosslinking strength and nanostructural complexity in supramolecular polymer networks need to be disentangled. In this thesis, a material toolkit was developed that allows for the formation of supramolecular polymer gels with model-network structure and for the consistent investigation of their mechanics, dynamics, and relaxation.

Their transient physical crosslinks render supramolecular polymer gels useful for a plethora of life science applications. However, these applications require the preparation of supramolecular gels in aqueous media under mild gelation conditions. Thus, supramolecular binding motifs that form interactions strong enough to withstand competitive hydrogen bonding with water along with biocompatible and water-soluble backbone polymers, such as polyglycerol and poly(ethylene glycol), were prepared in this thesis. Moreover, supramolecular gelation was combined with droplet-based microfluidics to prepare stimuli-responsive microgel particles. Such responsive microgels could be

used for drug delivery or for temporary cell encapsulation, allowing the cells to be studied and manipulated during encapsulation and then isolated and harvested on demand by decomposition of the microgel scaffolds.

An alternative approach to forming stimuli-responsive cell-laden microgels is based on covalent crosslinking of precursor polymers along with incorporation of degradable linkers into the polymer network. In particular, hydrolysis at acidic pH seems to have great potential for microgel degradation and release of encapsulated cells, because protons are known to play a major role in cellular communication and therefore encapsulated cells could actively degrade their hydrogel matrix. So far, the fabrication of acid-cleavable hydrogels involves gelation through free radicals or toxic catalysts, which would have detrimental effects on cell viability and proliferation. To overcome this limitation, one goal of this work was to synthesize polymeric precursors that can be crosslinked by radical- and catalyst-free bioorthogonal click chemistry and to combine this approach with microfluidic particle templating. The mechanical properties of the resulting microgels should be varied systematically to investigate their influence on the viability and proliferation of encapsulated cells.

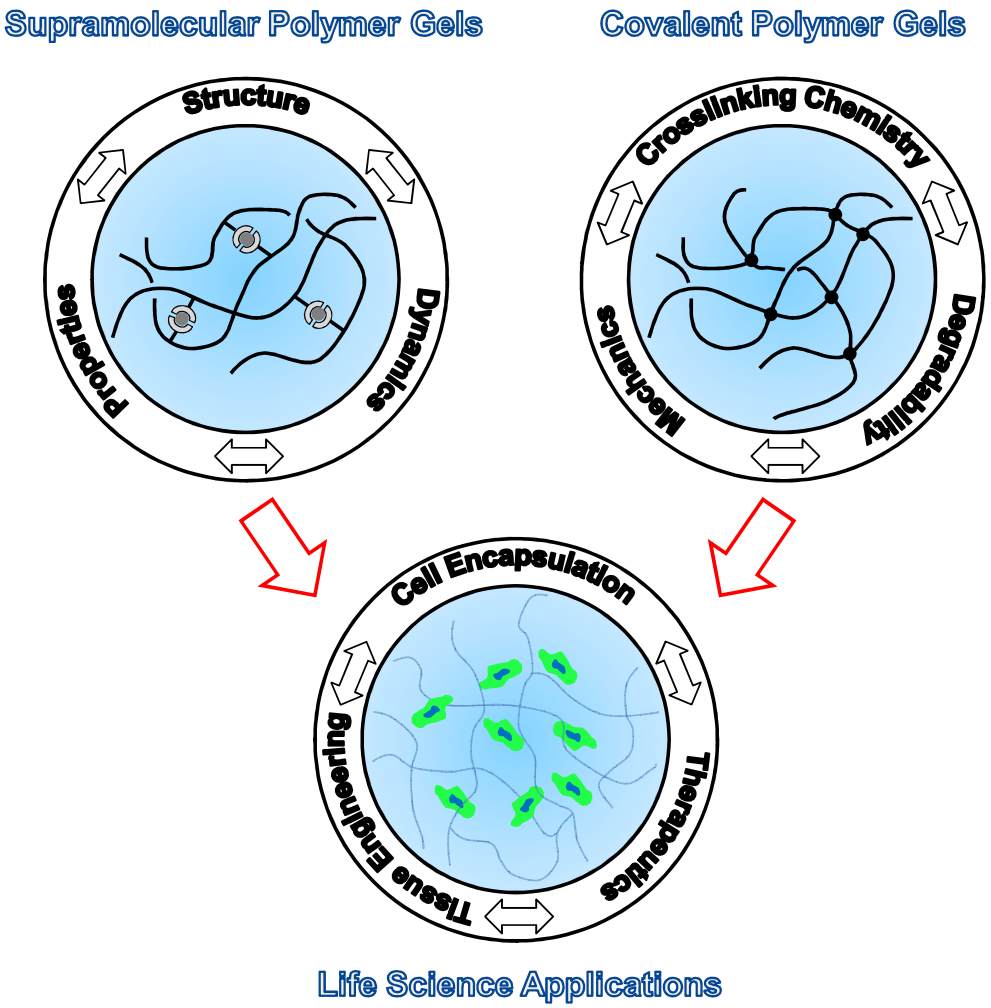
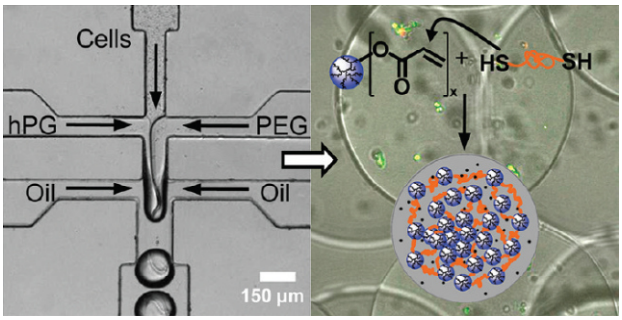



**Figure 19.** Schematics of the challenges to develop new polymeric materials for life science applications using supramolecular and covalent crosslinking chemistry.

# 3 Publications

## 3.1 Controlled Synthesis of Cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics




T. Rossow, J. A. Heyman, A. J. Ehrlicher, A. Langhoff, D. A. Weitz, R. Haag, and S. Seiffert, *J. Am. Chem. Soc.* **2012**, *134*, 4983–4989.
**DOI:** 10.1021/ja300460p
http://pubs.acs.org/doi/abs/10.1021/ja300460p

### Author contributions

**T. Rossow:** Project development, functionalization of polymeric precursors, construction of microfluidic devices, microfluidic templating and cell encapsulation, confocal laser scanning microscopy, determination of cell viabilities, preparation of the manuscript.

J. A. Heyman: Cell culturing.

A. J. Ehrlicher: Cell culturing.

A. Langhoff: Fluorescence Correlation Spectroscopy.

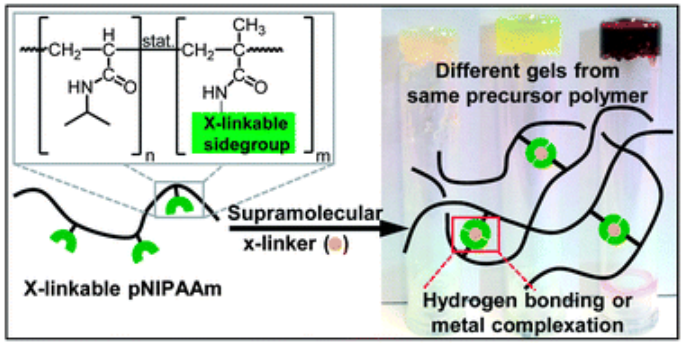
D. A. Weitz: Correction of the manuscript.

R. Haag: Conception and supervision of the work, correction of the manuscript.

S. Seiffert: Microfluidic-device engineering, confocal laser scanning microscopy, supervision, correction of the manuscript.

In this manuscript, micrometer-sized hydrogel particles that contain living cells were fabricated with exquisite control through the combination of droplet-based microfluidics with bio-orthogonal thiol–ene click chemistry. The microparticle gelation was achieved via the nucleophilic Michael addition of bioinert polymers such as dithiolated PEG macro-crosslinkers and acrylated hPG building blocks. This chemistry does not require any initiator and is therefore superior to existing techniques, in which microgel gelation is often achieved through harmful reactions with free radicals. We systematically varied the microgel properties through the use of PEG linkers with different molecular weights along with different concentrations of macromonomers to investigate the influence of these parameters on the viability and proliferation of encapsulated yeast cells. Moreover, the potential of this approach for the encapsulation of more sophisticated mammalian cells was demonstrated. For encapsulated fibroblasts and lymphoblasts excellent cell viabilities between 80% and 90% were achieved.

## 3.2 A Modular Construction Kit for Supramolecular Polymer Gels




T. Rossow, S. Hackelbusch, P. Van Assenbergh, and S. Seiffert, *Polym. Chem.* **2013**, *4*, 2515–2527.
**DOI:** 10.1039/C3PY00104K
http://pubs.rsc.org/en/Content/ArticleLanding/2013/PY/c3py00104k#!divAbstract

### Author contributions

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

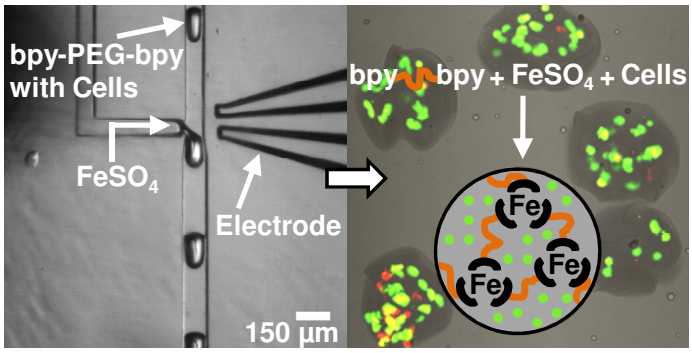
S. Hackelbusch: Polymer synthesis, rheology and ITC measurements, preparation of the manuscript.

P. Van Assenbergh: Rheology measurements.

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

In this manuscript, a construction kit for the preparation of supramolecular polymer gels was developed. First, linear chains of electrophilic methacryl-succinimidyl (MASI) modified poly(*N*-isopropylacrylamide) (pNIPAAm) were synthesized. Then, these polymers were modified in a modular fashion by replacing their electrophilic MASI units by nucleophilic amine-functionalized derivatives of custom, supramolecular crosslinkable functionalities. By this means, a set of pNIPAAm polymers that consist of exactly the same polymer backbone functionalized with different types of crosslinkable side groups could be prepared. Polymer chain crosslinking was achieved via hydrogen bonding or metal complexation by addition of low molecular weight crosslinkers that are complementary to the motifs on the polymer. The hydrogen bonding was based on diaminotriazine and maleimide, cyanuric acid and Hamilton wedges, and diaminotriazine and cyanuric acid; metal complexation was based on terpyridine and different metal salts. Using this approach we could form supramolecular networks of greatly varying rheological properties, each showing consistent and quantitative correlation between the gel mechanical properties and the binding strength of the respective constituent supramolecular crosslinking motifs.

## 3.3 Supramolecular Hydrogel Capsules Based on PEG: A Step Toward Degradable Biomaterials with Rational Design




T. Rossow, S. Bayer, R. Albrecht, C. C. Tzschucke, and S. Seiffert, *Macromol. Rapid Commun.* **2013**, *34*, 1401–1407.
**DOI:** 10.1002/marc.201300353
http://onlinelibrary.wiley.com/doi/10.1002/marc.201300353/abstract

### Author contributions

**T. Rossow:** Project development, polymer functionalization, microfluidic templating and cell encapsulation, confocal laser scanning microscopy, determination of cell viabilities, degradation studies, preparation of the manuscript.

S. Bayer: Construction of microfluidic devices, microfluidic templating and cell encapsulation, confocal laser scanning microscopy, cell culturing.

R. Albrecht: Synthesis of bipyridines, gelation studies, preparation of the manuscript.

C. C. Tzschucke: Correction of the manuscript.

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

In this manuscript, supramolecular microgel capsules based on poly(ethylene glycol) (PEG) were prepared by combining droplet-based microfluidics with supramolecular chain crosslinking. Linear PEG precursor polymers that carry bipyridine moieties on both chain termini were gelled by complexation to iron(II) ions. To investigate the biocompatibility of the microgels, living mammalian cells were encapsulated within them. The microgel elasticity was controlled by using PEG precursors of different molecular weights along with different concentrations of these precursors. By this means, the microgel matrix properties could be optimized to exceed cell viabilities of more than 90%. Reversion of the supramolecular polymer crosslinking by addition of competitive ligands allowed the microcapsules to be degraded at mild conditions with no effect on the viability of the encapsulated and released cells. This approach is an important step toward a new platform for storing, studying, and manipulating cells within artificial extracellular matrixes, whereas controlled cell release allows for subsequent cell harvesting and further studying by standard and established biological methods.

## 3.4 Chain Dynamics in Supramolecular Polymer Networks

S. Hackelbusch, T. Rossow, P. Van Assenbergh, and S. Seiffert, *Macromolecules* **2013**, *46*, 6273–6286.
**DOI:** 10.1021/ma4003648
http://pubs.acs.org/doi/abs/10.1021/ma4003648

### Author contributions

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

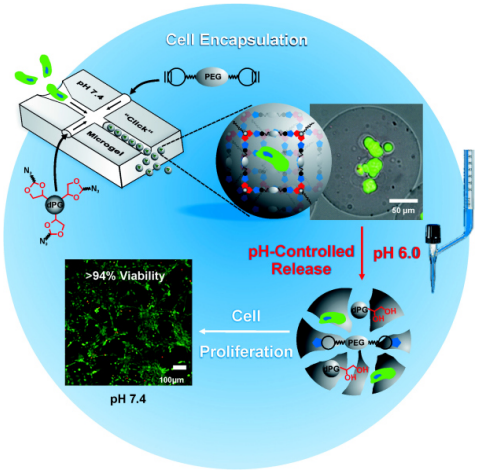
**T. Rossow:** Synthesis of supramolecular crosslinkable motifs.

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 form supramolecular polymer networks that exhibit greatly varying strength of transient chain crosslinking but that are all derived from the very same precursor polymer. This strategy allowed the impact of the strength of transient chain crosslinking on the network dynamics and mechanics to be studied with high consistency. We evaluated the diffusive mobility of labeled tracer chains within these transient networks. Additionally, we probed the macroscopic mechanics of the same supramolecular networks by shear rheology. Our results revealed that the concentration dependence of the tracer-chain diffusivity is in agreement with theoretical predictions derived from the “sticky reptation” model by Rubinstein and Semenov, provided the chain association is stronger than a certain threshold. By contrast, at weaker chain association classical semidilute-solution-type chain dynamics were observed. In a longer perspective, this knowledge could be a basis for sophisticated modeling of the self-healing properties of supramolecular polymer networks.

## 3.5 A Microgel Construction Kit for Bioorthogonal Encapsulation and pH-Controlled Release of Living Cells




D. Steinhilber,* T. Rossow,* S. Wedepohl, F. Paulus, S. Seiffert, and R. Haag, *Angew. Chem. Int. Ed.* **2013**, *52*, 13538–13543; *Angew. Chem.* **2013**, *125*, 13780–13785. [*equal contribution]
**DOI:** 10.1002/anie.201308005
http://onlinelibrary.wiley.com/doi/10.1002/anie.201308005/abstract

### Author contributions

D. Steinhilber: Project development, synthesis of azide-functionalized macromonomers, electrophilic cyclooctynes, and homobifunctional cyclooctyne-crosslinkers, gelation studies, preparation of the manuscript.

**T. Rossow:** Project development, synthesis of PEG-diamine, microfluidic templating and cell encapsulation, pH-controlled microgel degradation and cell release, preparation of the manuscript.

S. Wedepohl: Cell culturing, correction of the manuscript.

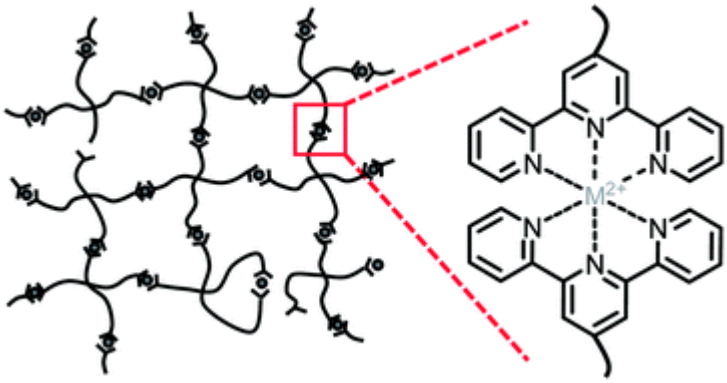
F. Paulus: Polyglycerol synthesis.

S. Seiffert: Correction of the manuscript.

R. Haag: Conception and supervision of the work, correction of the manuscript.

In this manuscript, a new microgel construction kit was developed serving for the bioorthogonal encapsulation of living cells by strain-promoted azide–alkyne cycloaddition (SPAAC) and the programmed cell release triggered by benzacetal hydrolysis. We combined this chemistry with droplet-based microfluidics to generate stimuli-responsive mammalian-cell-laden microgel particles. Poly(ethylene glycol)dicyclooctyne and dendritic poly(glycerol azide) served as bioinert hydrogel precursors to avoid non-specific hydrogel–cell interactions. The encapsulated mammalian cells could be cultured inside the microgels with full retention of their viability. The use of different substituted benzacetals as pH-cleavable linkers on the dendritic building block allowed precise control of the microgel degradation kinetics in the interesting pH range between 4.5 and 7.4. By this means, a pH-controlled release of the encapsulated cells was achieved upon demand with no effect on cell viability, spreading, and proliferation. As a result, this approach has the potential to advance the understanding of cellular survival in artificial 3D matrix environments.

## 3.6 Supramolecular Polymer Gels with Potential Model-Network Structure




T. Rossow and S. Seiffert, *Polym. Chem.* **2014**, *5*, 3018–3029.
**DOI:** 10.1039/C3PY01692G
http://pubs.rsc.org/en/Content/ArticleLanding/2014/PY/c3py01692g#!divAbstract

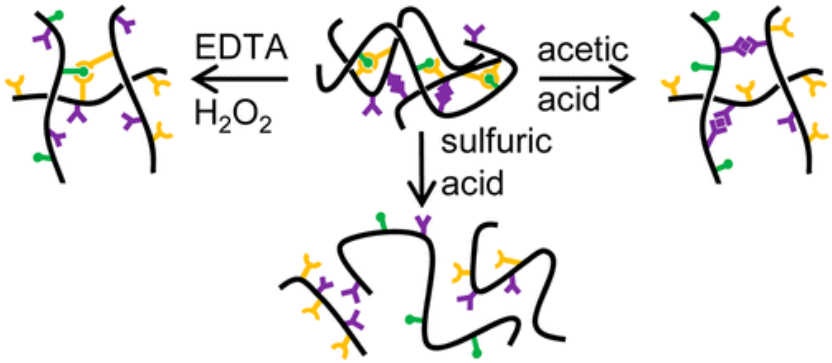
### Author contributions

**T. Rossow:** Project development, synthesis, rheology and ITC measurements, data analysis, preparation of the manuscript.

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

In this manuscript, a modular toolkit to form supramolecular polymer networks was presented that allows the control of both the strength of transient chain crosslinking and the polymer network topology. Our approach was based on transition-metal mediated linking of star-shaped poly(ethylene glycol) building blocks that were end-capped with terpyridine moieties. This allowed supramolecular networks of greatly varying strengths of transient interlinkage to be prepared by a modular choice of the linking metal ion and the surrounding solvent. By this means, a set of different supramolecular polymer gel networks was synthesized with mechanical properties that are quantitatively related to the strength of their constituent crosslinking complexes. Static light scattering revealed just minor nanometer-scale polymer network inhomogeneity in some of the gels, whereas others exhibited non-negligible nanostructural heterogeneity. In the latter gels, the mechanical strength and resistance to relaxation was found to be greater than expected, indicating clustering of supramolecular crosslinks to be a mechanism of enforcement.

## 3.7 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]
**DOI:** 10.1021/ma5008573
http://pubs.acs.org/doi/abs/10.1021/ma5008573

### Author contributions

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

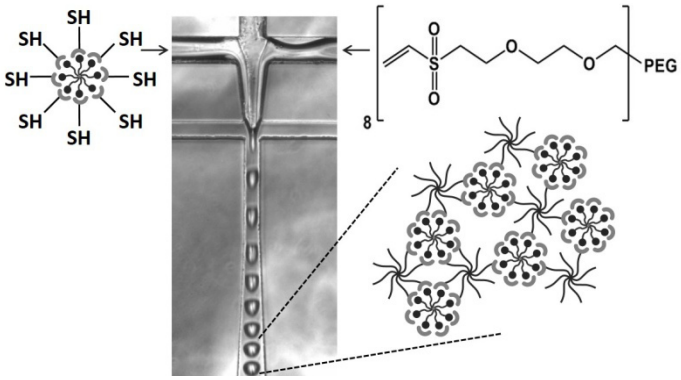
**T. Rossow:** Project conception and development, synthesis of supramolecular crosslinkable motifs and of linear polyglycerol, polymer functionalization, rheology measurements, de-crosslinking studies, preparation of the manuscript.

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

S. Seiffert: Supervision, correction of the manuscript.

In this manuscript, we presented an approach to prepare multiresponsive supramolecular hydrogels crosslinked by either hydrogen bonding, metal complexation, or both. We employed strain-promoted azide–alkyne cycloaddition to functionalize biocompatible linear polyglycerol in the side chains with cyanurate and diaminotriazine moieties that form a multipoint hydrogen-bonded array, along with terpyridine moieties that form bivalent metal complexes with iron(II) ions. By this means, supramolecular hydrogels could be formed at mild conditions in water that remained stable for several weeks. The orthogonal reversibility of the crosslinks allowed the hydrogels to be selectively degelled by orthogonal stimulation. Acetic acid was used to solely disrupt the hydrogen bonds, whereas the use of EDTA along with hydrogen peroxide solely disrupted the iron complexes by oxidation of iron(II) to iron(III) and capture of the liberated iron ions. Sulfuric acid could be employed to simultaneously disrupt both these types of interactions.

## 3.8 Microfluidic Synthesis of Pharmacologically Responsive Supramolecular Biohybrid Microgels




D. Hövermann,* T. Rossow,* R. J. Gübeli, S. Seiffert, and W. Weber, *Macromol. Biosci.* **2014**, *in print*. [*equal contribution]
**DOI:** 10.1002/mabi.201400342
http://onlinelibrary.wiley.com/doi/10.1002/mabi.201400342/abstract

### Author contributions

D. Hövermann: Biological evaluation of the microgels, preparation of the manuscript.

**T. Rossow:** Microfluidic templating, preparation of the manuscript.

R. J. Gübeli: Production and purification of cysteine-tagged scFv.

S. Seiffert: Preparation and submission of the manuscript.

W. Weber: Conception and supervision of the work, preparation of the manuscript.

In this manuscript, we developed a generically applicable method for the synthesis of micrometer-scale, injection-ready biohybrid materials. We used droplet-based microfluidics to generate monodisperse pre-microgel fluid droplets, wherein which fluorescein-modified 8-arm poly(ethylene glycol) was reacted with a thiol-functionalized humanized anti-fluorescein single chain antibody fragment and vinylsulfone-functionalized 8-arm poly(ethylene glycol), resulting in the formation of stable, narrowly dispersed supramolecular microgels (30 and 150 µm diameter). We demonstrated that these biohybrid microgels can change their mechanical properties in response to pharmacological cues. The addition of free fluorescein to the microgels results in a weakening of their hydrogel structure, eventually leading to its disintegration. This method of formation of pharmacologically responsive biohybrid hydrogels in an injection-ready formulation is a pioneering example of a general approach for the synthesis of biohybrid hydrogel-based drug depots for biomedical applications.

# 4 Summary and Conclusions

In this thesis, a modular construction kit for supramolecular polymer gels has been prepared by use of linear chains of electrophilic methacryl-succinimidyl (MASI) modified poly(*N*-isopropylacrylamide). The MASI units were replaced by nucleophilic substitution with amine-functionalized derivatives of supramolecular crosslinkable motifs. By this means, a set of pNIPAAm polymers that consist of exactly the same polymer backbone functionalized with different types of supramolecular crosslinkable side groups has been synthesized. Since the pNIPAAm is soluble in a variety of solvents, gels can be prepared and studied at various solvent conditions. Polymer chain crosslinking is achieved via hydrogen bonding or metal complexation by addition of low molecular weight crosslinkers that are complementary to the motifs on the polymer. In all cases, the mechanical properties of the resulting supramolecular gels are determined by the strength of supramolecular association, as revealed quantitatively by oscillatory shear rheology and isothermal titration calorimetry. By this means, supramolecular networks of greatly varying mechanical strength, from low viscous liquids to highly elastic gels, could be prepared.

Additionally, the present toolkit allows further functionalization of the precursors with amine-functionalized fluorescent markers to probe the polymer networks by fluorescence-based imaging or tracking techniques. We followed this approach and studied these supramolecular networks by probing the micrometer-scale mobility of fluorescently tagged linear chains that diffuse through them. As a result, at a certain threshold strength of association the concentration dependence of the tracer-chain diffusivity is in agreement with theoretical predictions derived from the ‘sticky reptation’ model by Rubinstein and Semenov.

Although our investigations demonstrated the utility of our modular toolkit, they also revealed a persistent limitation: complex long-term relaxation is observed that cannot be clearly related to theoretical modeling. We attributed this finding to irregular and inhomogeneous network structure caused by the polydispersity of both the precursor chain molecular weight and the side group substitution pattern. As a consequence, we developed a material toolkit to form supramolecular polymer networks with different crosslinking strengths that have the potential to exhibit close to regular and determined nanometer-scale network topologies. For this purpose, narrowly distributed tetra-arm poly(ethylene glycol) precursors were end-capped with terpyridine moieties. Different transition metal ions such as $Mn^{2+}$, $Zn^{2+}$, and $Co^{2+}$ were used to form transient networks with different strengths of connectivity in a variety of different solvents. Since the polymer network mesh size is determined by the molecular weight of the arms of the precursor chains and since this molecular weight is regular and narrowly disperse, networks of highly regular topology can be obtained, as determined by static light scattering.

As hydrogel-based drug depots for biomedical applications, we developed pharmacologically responsive supramolecular biohybrid microgels. For this purpose, a generically applicable method for the synthesis of micrometer-scale, injection-ready biohybrid materials was devised. Droplet-based microfluidics was used to generate monodisperse pre-microgel fluid droplets, wherein which fluorescein-modified 8-arm PEG was reacted with a thiol-functionalized humanized anti-fluorescein single chain antibody fragment and vinylsulfone-functionalized 8-arm PEG. This resulted in the formation of stable, narrowly dispersed supramolecular microgels of 30 and 150 µm in diameter. The addition of free fluorescein to these microgels induced a weakening of their hydrogel structure, eventually leading to its disintegration.

For the encapsulation of living cells into reversibly crosslinked microgel particles, again, droplet-based microfluidics and supramolecular gelation have been combined. Linear PEG precursor polymers that carry bipyridine moieties on both chain termini were gelled by complexation to iron(II) ions. By using PEG precursors of different molecular weights at different concentrations the microgel elasticity can be controlled and thereby the cell viabilities have been optimized to exceed 90%. The microgels are degradable by addition of competitive ligands within 1.5 h under very mild conditions, with no effect on the viability of the encapsulated and released cells. Although this approach is an important step toward a new platform for storing, studying, and manipulating cells within artificial extracellular matrixes and releasing them subsequently, it is not suitable for long-term applications due to microgel auto-degradation within timespans of just several hours. A strategy to overcome this limitation is to increase the density of reversibly associating groups, along with increasing the extent of chain entanglement; both require polymer side-group functionalization rather than polymer end-group functionalization.

Thus, we developed a supramolecular polymer toolkit based on biocompatible linear polyglycerol that is functionalized with orthogonal supramolecular crosslinkable side groups. Strain-promoted azide−alkyne cycloaddition was employed to functionalize the polymer backbone with cyanurate and diaminotriazine moieties that form a multipoint hydrogen-bonded array, along with terpyridine moieties that form bivalent metal complexes with iron(II) ions. By this type of orthogonal crosslinking, supramolecular hydrogels can be formed at mild conditions that remain stable for several weeks. The orthogonal reversibility of the crosslinks allows the hydrogels to be selectively de-crosslinked by orthogonal stimulation. Whereas addition of metal chelators has no impact on the hydrogen-bonding crosslinking in these systems, it reverses the metal-complexation crosslinking. Conversely, addition of acetic acid reverses the hydrogen bonding, but has no influence on the metal complexation. Their responsiveness and reversibility along with the biocompatibility of the lPG backbone polymer render this class of hydrogels promising for use in temporary cell encapsulation and controlled cell release in future work.

As an alternative to supramolecular crosslinking to generate stimuli-responsive cell-laden microgels, we employed covalent crosslinking along with incorporation of degradable linkers into the polymer network. We combined droplet microfluidic templating with bio-orthogonal thiol−ene click chemistry to fabricate monodisperse, cell-laden microgel particles in which the encapsulated mammalian cells exhibit excellent viabilities of up to 90%. In this approach, micro-droplet gelation is achieved via the nucleophilic Michael addition of dithiolated PEG macro-crosslinkers to acrylated hPG building blocks. By systematic variation of the microgel mechanical properties, their influence on the viability and proliferation of encapsulated cells has been probed. Degradation of the microgel particles through hydrolysis of the ester bond close to the thioether bond has been observed over a time of several weeks, but has not been precisely controllable or tunable.

To overcome this limitation we designed a new microgel construction kit for the bioorthogonal encapsulation of living cells using strain-promoted azide–alkyne cycloaddition for crosslinking along with acid-labile benzacetal linkers for the programmed cell release. The variation of the substitution pattern of the benzacetals allows precise control of the microgel degradation kinetics in the interesting pH range between 4.5 and 7.4. The encapsulated cells could be cultured inside the microgels with full retention of their viability and the subsequent microgel degradation had no detrimental effect on the encapsulated and released cells. Besides temporary cell encapsulation, this construction kit has potential for the stabilization and controlled release of many other therapeutic relevant biological systems such as proteins, genes, and even bacteria.

# 5 Zusammenfassung und Fazit

In dieser Arbeit wurde ein modulares Baukastensystem für die Herstellung von supramolekularen Polymergelen entwickelt. Dazu wurde zunächst lineares Poly(*N*-isopropylacrylamid) (PNIPAAm) hergestellt, das elektrophile Methacryl-Succinimidyl-Einheiten (MASI) enthielt. Diese MASI-Einheiten konnten dann durch nukleophile Substitution mit Amin-funktionalisierten Derivaten supramolekular vernetzbarer Motive umgesetzt werden. Auf diese Weise konnte ein Satz von Polymeren hergestellt werden, die aus ein und demselben Rückgrat bestehen, aber unterschiedliche Typen supramolekular vernetzbarer Seitengruppen enthalten. Da PNIPAAm in einer Vielzahl von verschiedenen Lösungsmitteln löslich ist, konnten auch die entsprechenden supramolekularen Netzwerke in verschieden Lösungsmitteln hergestellt und untersucht werden. Die Polymerketten wurden über Wasserstoffbrückenbindungen oder Metallkomplexierung vernetzt, indem niedermolekulare Vernetzer hinzugegeben wurden, die komplementär zu den supramolekularen Motiven des Polymers sind. In allen untersuchten Fällen werden die mechanischen Eigenschaften der supramolekularen Netzwerke durch die Stärke der supramolekularen Wechselwirkung bestimmt, was quantitativ mittels oszillatorischer Scherrheologie und isothermaler Titrationskalorimetrie gezeigt werden konnte. Auf diese Weise konnten supramolekulare Netzwerke stark unterschiedlicher mechanischer Stärke, von niedrig viskosen Flüssigkeiten bis hin zu hoch elastischen Gelen, hergestellt werden.

Darüber hinaus erlaubt der vorliegende Materialbaukasten die Funktionalisierung der Polymere mit Amin-funktionalisierten fluoreszierenden Markern, um die Polymernetzwerke mittels fluoreszenzbasierten Bildgebungsverfahren untersuchen zu können. Wir haben diesen Ansatz verfolgt und die Mobilität von fluoreszenzmarkierten linearen Ketten in den supramolekularen Polymernetzwerken untersucht. Ab einer bestimmten Bindungsstärke der supramolekularen Motive verhielt sich die Konzentrationsabhängigkeit der Kettendiffusivität in Übereinstimmung mit theoretischen Vorhersagen des ‚sticky reptation' Modells von Rubinstein und Semenov.

Wenngleich all diese Untersuchungen die Nützlichkeit unseres modularen Baukastensystems gezeigt haben, wurden gleichzeitig auch seine Grenzen aufgedeckt: Eine komplexe Langzeit-Relaxation wurde beobachtet, die nicht eindeutig mit theoretischen Modellen beschrieben werden kann. Wir haben diese Beobachtung auf eine uneinheitliche und inhomogene Netzwerkstruktur zurückgeführt, die sowohl von der Polydispersität der Vorläuferpolymere als auch von deren uneinheitlicher Seitengruppenvernetzung verursacht wird. Als Konsequenz hieraus haben wir ein weiteres Baukastensystem entwickelt, mit dem man wiederum supramolekulare Polymernetzwerke unterschiedlicher Stärke erzeugen kann, die jedoch eine einheitliche und definierte nanometerskalige Netzwerktopologie aufweisen. Hierfür wurden engverteilte Vierarm-Poly(ethylenglykol)-Vorläufer an ihren Kettenenden mit Terpyridin-Motiven funktionalisiert. Unterschiedliche Übergangsmetallionen wie $Mn^{2+}$, $Zn^{2+}$, und $Co^{2+}$ wurden benutzt, um

supramolekulare Netzwerke unterschiedlicher Vernetzungsstärke in verschiedenen Lösungsmitteln herzustellen. Da die Maschenweite der Polymernetzwerke durch das Molekulargewicht der Arme der Vorläuferpolymere bestimmt wird und da dieses Molekulargewicht einheitlich und engverteilt ist, können extrem homogene Netzwerke erhalten werden, wie mittels statischer Lichtstreuung gezeigt werden konnte.

Als hydrogelbasierte Wirkstoffdepots für biomedizinische Anwendungen haben wir pharmakologisch-responsive supramolekulare Biohybrid-Mikrogele hergestellt. Dazu wurde eine generell einsetzbare Methode für die Herstellung von mikrometerskaligen, injizierbaren Biohybridmikrogelen entwickelt. Tröpfchenbasierte Mikrofluidik wurde benutzt, um monodisperse Tröpfchen herzustellen, in denen fluorescein-modifiziertes Achtarm-PEG mit einem Thiol-funktionalisierten humanisierten Anti-Fluorescein scFv-Fragment und Vinylsulfon-funktionalisiertem Achtarm-PEG reagierten. Als Folge wurden stabile, engverteilte supramolekulare Mikrogele von 30 und 150 µm Durchmesser erhalten. Die Zugabe von freiem Fluorescein zu diesen Mikrogelen führte zur Schwächung ihrer Hydrogelstruktur bzw. schließlich zu deren Auflösung.

Für die Verkapselung von lebenden Zellen in reversibel vernetzte Mikrogelpartikel wurden die tröpfchenbasierte Mikrofluidik und die supramolekulare Vernetzungschemie wiederum miteinander kombiniert. Lineare PEG Vorläuferpolymere wurden an ihren beiden Kettenenden mit Bipyridinen funktionalisiert und durch Komplexierung mit Eisen(II)-ionen zu Mikrogelen vernetzt. Durch Wahl von PEG-Polymeren unterschiedlichen Molekulargewichts bei unterschiedlichen Polymerkonzentrationen konnte die Mikrogelelastizität eingestellt werden. Dadurch gelang es, die Mikrogeleigenschaften für die Zellverkapselung zu optimieren und Zellvitabilitäten von über 90% zu erreichen. Die erhaltenen Mikrogele können durch Zugabe von kompetitiven Liganden innerhalb von 1,5 Stunden unter milden Bedingungen aufgelöst werden. Der Abbauprozess der Partikel hat keine schädlichen Auswirkungen auf die Zellvitabilität der verkapselten und freigesetzten Zellen. Dieser Ansatz ist ein wichtiger Schritt hin zu einer neuen Materialplattform, die es erlaubt, Zellen in einer künstlichen extrazellulären Matrix zu verkapseln, zu untersuchen, zu manipulieren und anschließend wieder freizusetzen. Allerdings ist der vorliegende Ansatz nicht für Langzeitanwendungen geeignet, da die Mikrogele innerhalb mehrerer Stunden durch Autoabbau aufgelöst werden. Diese Einschränkung könnte überwunden werden, indem die Dichte vernetzbarer Gruppen erhöht und die Kettenverschlaufung verstärkt wird. Hierzu müssten die polymeren Vorläufer in ihren Seitenketten funktionalisiert werden und nicht nur an ihren Enden.

Um dieses Ziel zu erreichen, wurde lineares Polyglycerol synthetisiert und an den Seitenketten mit orthogonal vernetzbaren supramolekularen Motiven funktionalisiert. Die spannungsvermittelte Azid–Alkin Zykloaddition wurde verwendet, um das Polymer einerseits mit Cyanurat- und Diaminotriazin-Einheiten zu funktionalisieren, die zusammen eine komplexe Anordnung von

Wasserstoffbrücken ausbilden, und andererseits mit Terpyridin-Einheiten, die bivalente Metallkomplexe mit Eisen(II)-ionen eingehen. Diese Art der orthogonalen Vernetzung ermöglicht die Bildung von supramolekularen Hydrogelen unter milden Bedingungen, wobei die erhaltenen Gele über eine exzellente Langzeitstabilität von mehreren Wochen verfügen. Gleichzeitig ermöglicht die orthogonale Reversibilität der Netzknoten die Hydrogele durch orthogonale Stimulierung selektiv zu entnetzen. Die Zugabe von Metall-Chelatoren hat keinen Einfluss auf die Wasserstoffbrückenvernetzung, jedoch führt sie zur Auflösung der Metall-komplexierten Vernetzung. Umgekehrt führt die Zugabe von Essigsäure zur Auflösung der Wasserstoffbrückenbindungen, ohne einen Einfluss auf die Metallkomplexierung zu haben. Ihre Responsivität und Reversibilität, vereint mit der Biokompatibilität von linearem Polyglycerol, machen diese Hydrogele vielversprechend für die zukünftige Verwendung in der temporären Zellverkapselung und der kontrollierten Zellfreisetzung.

Als Alternative der supramolekularen Vernetzung zur Herstellung von stimuli-responsiven zellbeladenen Mikrogelen haben wir die kovalente Vernetzung in Kombination mit abbaubaren Linkern verwendet. Hierzu wurde die tröpfchenbasierte Mikrofluidik mit bioorthogonaler Thiol–En Click-Chemie gekoppelt und es konnten mit Säugetierzellen beladene Mikrogele hergestellt werden, in denen die verkapselten Zellen exzellente Überlebensraten von über 90% aufwiesen. In diesem Ansatz wird die Tröpfchengelierung durch die nukleophile Michael-Addition von PEG-dithiol-Makromonomeren und acrylierten hPG-Bausteinen erreicht. Durch systematische Variation der mechanischen Eigenschaften der Mikrogelpartikel wurde ihr Einfluss auf die Zellvitabilität und die Zellvermehrung untersucht. Desweiteren wurde ein Abbau der Mikrogelpartikel aufgrund der Hydrolyse der Esterbindung nahe der Thioetherbindung in einem Zeitraum von einigen Wochen beobachtet. Allerdings konnte dieser Abbau nicht präzise gesteuert und eingestellt werden.

Zur Überwindung dieser Einschränkung haben wir einen neuartigen Mikrogelbaukasten entwickelt, mit dem lebende Zellen durch Vernetzung mit der spannungsvermittelten Azid–Alkin Zykloaddition verkapselt und durch säurelabile Benzacetallinker im Polymernetzwerk wieder freigesetzt werden können. Durch Änderung des Substitutionsmusters der Benzacetale kann die Kinetik des Mikrogelabbaus im interessanten pH-Bereich von 4,5 bis 7,4 exakt gesteuert werden. Die verkapselten Zellen können in den Mikrogelen unter vollständiger Aufrechterhaltung ihrer Vitabilität kultiviert werden und auch der darauffolgende Mikrogelabbau hat keine negativen Auswirkungen auf die verkapselten und freigesetzten Zellen. Neben der temporären Zellverkapselung hat dieser Mikrogelbaukasten das Potential für die Stabilisierung und kontrollierte Freisetzung vieler anderer therapeutisch wichtiger biologischer Systeme, wie z. B von Proteinen, Genen und sogar Bakterien.

# 6 References


1. Kopeček, J. *Biomaterials* **2007**, *28*, 5185–5192.
2. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27–46.
3. Kopeček, J.; Yang, J. *Polym. Int.* **2007**, *56*, 1078–1098.
4. Wichterle, O.; Lim, D. *Nature* **1960**, *185*, 117–118.
5. Hoare, T. R.; Kohane, D. S. *Polymer* **2008**, *49*, 1993–2007.
6. Vashist, A.; Vashist, A.; Gupta, Y. K.; Ahmad, S. *J. Mater. Chem. B* **2014**, *2*, 147–166.
7. Censi, R.; Di Martino, P.; Vermonden, T.; Hennink, W. E. *J. Control. Release* **2012**, *161*, 680–692.
8. Magnusson, J. P.; Saeed, A. O.; Fernandez-Trillo, F.; Alexander, C. *Polym. Chem.* **2011**, *2*, 48–59.
9. Kabiri, K.; Omidian, H.; Zohuriaan-Mehr, M. J.; Doroudiani, S. *Polym. Compos.* **2011**, *32*, 277–289.
10. Lee, K. Y.; Mooney, D. J. *Chem. Rev.* **2001**, *101*, 1869–1880.
11. Drury, J. L.; Mooney, D. J. *Biomaterials* **2003**, *24*, 4337–4351.
12. Langer, R. *Acc. Chem. Res.* **1999**, *33*, 94–101.
13. Nicodemus, G. D.; Bryant, S. J. *Tissue Eng. Part B Rev.* **2008**, *14*, 149–65.
14. Thiele, J.; Ma, Y.; Bruekers, S. M. C.; Ma, S.; Huck, W. T. S. *Adv. Mater.* **2014**, *26*, 125–148.
15. Annabi, N.; Tamayol, A.; Uquillas, J. A.; Akbari, M.; Bertassoni, L. E.; Cha, C.; Camci-Unal, G.; Dokmeci, M. R.; Peppas, N. A.; Khademhosseini, A. *Adv. Mater.* **2014**, *26*, 85–124.
16. Slaughter, B. V.; Khurshid, S. S.; Fisher, O. Z.; Khademhosseini, A.; Peppas, N. A. *Adv. Mater.* **2009**, *21*, 3307–3329.
17. Putnam, A. J.; Mooney, D. J. *Nat. Med.* **1996**, *2*, 824–826.
18. Jen, A. C.; Wake, M. C.; Mikos, A. G. *Biotechnol. Bioeng.* **1996**, *50*, 357–364.
19. Vert, M.; Doi, Y.; Hellwich, K.-H.; Hess, M.; Hodge, P.; Kubisa, P.; Rinaudo, M.; Schué, F., Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). In *Pure Appl. Chem.*, 2012; Vol. 84, p 377.
20. Goh, C. H.; Heng, P. W. S.; Chan, L. W. *Carbohydr. Polym.* **2012**, *88*, 1–12.
21. Lehr, C.-M.; Bouwstra, J. A.; Schacht, E. H.; Junginger, H. E. *Int. J. Pharm.* **1992**, *78*, 43–48.
22. Baier Leach, J.; Bivens, K. A.; Patrick Jr, C. W.; Schmidt, C. E. *Biotechnol. Bioeng.* **2003**, *82*, 578–589.
23. Eyrich, D.; Brandl, F.; Appel, B.; Wiese, H.; Maier, G.; Wenzel, M.; Staudenmaier, R.; Goepferich, A.; Blunk, T. *Biomaterials* **2007**, *28*, 55–65.
24. White, M. L. *J. Phys. Chem.* **1960**, *64*, 1563–1565.
25. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. *Angew. Chem. Int. Ed.* **2010**, *49*, 6288–6308.
26. Metters, A.; Hubbell, J. *Biomacromolecules* **2004**, *6*, 290–301.
27. DiRamio, J. A.; Kisaalita, W. S.; Majetich, G. F.; Shimkus, J. M. *Biotechnol. Prog.* **2005**, *21*, 1281–1288.
28. Lutolf, M. P.; Lauer-Fields, J. L.; Schmoekel, H. G.; Metters, A. T.; Weber, F. E.; Fields, G. B.; Hubbell, J. A. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5413–5418.
29. Zustiak, S. P.; Leach, J. B. *Biomacromolecules* **2010**, *11*, 1348–1357.
30. García, A. *Ann. Biomed. Eng.* **2014**, *42*, 312–322.
31. Adzima, B. J.; Tao, Y.; Kloxin, C. J.; DeForest, C. A.; Anseth, K. S.; Bowman, C. N. *Nat. Chem.* **2011**, *3*, 256–259.
32. Elbert, D. L.; Hubbell, J. A. *Biomacromolecules* **2001**, *2*, 430–441.
33. Sakai, T.; Matsunaga, T.; Yamamoto, Y.; Ito, C.; Yoshida, R.; Suzuki, S.; Sasaki, N.; Shibayama, M.; Chung, U.-i. *Macromolecules* **2008**, *41*, 5379–5384.
34. Roberts, J. J.; Bryant, S. J. *Biomaterials* **2013**, *34*, 9969–9979.
35. Lei, J.; Mayer, C.; Freger, V.; Ulbricht, M. *Macromol. Mater. Eng.* **2013**, *298*, 967–980.

36. Falender, J. R.; Yeh, G. S. Y.; Mark, J. E. *J. Am. Chem. Soc.* **1979**, *101*, 7353–7356.
37. Falender, J. R.; Yeh, G. S. Y.; Mark, J. E. *Macromolecules* **1979**, *12*, 1207–1209.
38. Mark, J. E.; Tang, M. Y. *J. Polym. Sci., Polym. Phys. Ed.* **1984**, *22*, 1849–1855.
39. Tang, M. Y.; Mark, J. E. *Macromolecules* **1984**, *17*, 2616–2619.
40. Beinert, G.; Belkebir-Mrani, A.; Herz, J.; Hild, G.; Rempp, P. *Faraday Discuss. Chem. Soc.* **1974**, *57*, 27–34.
41. Akagi, Y.; Matsunaga, T.; Shibayama, M.; Chung, U.-i.; Sakai, T. *Macromolecules* **2009**, *43*, 488–493.
42. Sakai, T. *React. Funct. Polym.* **2013**, *73*, 898–903.
43. Peppas, N. A.; Hilt, J. Z.; Khademhosseini, A.; Langer, R. *Adv. Mater.* **2006**, *18*, 1345–1360.
44. Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Discov.* **2003**, *2*, 214–221.
45. Armstrong, J. K.; Hempel, G.; Koling, S.; Chan, L. S.; Fisher, T.; Meiselman, H. J.; Garratty, G. *Cancer* **2007**, *110*, 103–111.
46. Calderón, M.; Quadir, M. A.; Sharma, S. K.; Haag, R. *Adv. Mater.* **2010**, *22*, 190–218.
47. Steinhilber, D.; Seiffert, S.; Heyman, J. A.; Paulus, F.; Weitz, D. A.; Haag, R. *Biomaterials* **2011**, *32*, 1311–1316.
48. Oudshoorn, M. H. M.; Rissmann, R.; Bouwstra, J. A.; Hennink, W. E. *Biomaterials* **2006**, *27*, 5471–5479.
49. Sisson, A. L.; Papp, I.; Landfester, K.; Haag, R. *Macromolecules* **2008**, *42*, 556–559.
50. 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. USA* **2010**, *107*, 19679–19684.
51. Fischer, W.; Calderón, M.; Schulz, A.; Andreou, I.; Weber, M.; Haag, R. *Bioconj. Chem.* **2010**, *21*, 1744–1752.
52. Khandare, J.; Calderon, M.; Dagia, N. M.; Haag, R. *Chem. Soc. Rev.* **2012**, *41*, 2824–2848.
53. Calderón, M.; Welker, P.; Licha, K.; Fichtner, I.; Graeser, R.; Haag, R.; Kratz, F. *J. Control. Release* **2011**, *151*, 295–301.
54. Steinhilber, D.; Witting, M.; Zhang, X.; Staegemann, M.; Paulus, F.; Friess, W.; Küchler, S.; Haag, R. *J. Control. Release* **2013**, *169*, 289–295.
55. Weinhart, M.; Grunwald, I.; Wyszogrodzka, M.; Gaetjen, L.; Hartwig, A.; Haag, R. *Chem. Asian J.* **2010**, *5*, 1992–2000.
56. Chapanian, R.; Constantinescu, I.; Rossi, N. A. A.; Medvedev, N.; Brooks, D. E.; Scott, M. D.; Kizhakkedathu, J. N. *Biomaterials* **2012**, *33*, 7871–7883.
57. Kainthan, R. K.; Brooks, D. E. *Biomaterials* **2007**, *28*, 4779–4787.
58. Haag, R.; Sunder, A.; Stumbé, J.-F. *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955.
59. Sunder, A.; Hanselmann, R.; Frey, H.; Mülhaupt, R. *Macromolecules* **1999**, *32*, 4240–4246.
60. Thomas, A.; Müller, S. S.; Frey, H. *Biomacromolecules* **2014**, *15*, 1935–1954.
61. Vandenberg, E. J. *J. Polym. Sci. Polym. Chem. Ed.* **1985**, *23*, 915–949.
62. Tokar, R.; Kubisa, P.; Penczek, S.; Dworak, A. *Macromolecules* **1994**, *27*, 320–322.
63. Dworak, A.; Walach, W.; Trzebicka, B. *Macromol. Chem. Phys.* **1995**, *196*, 1963–1970.
64. Paulus, F.; Weiss, M. E. R.; Steinhilber, D.; Nikitin, A. N.; Schütte, C.; Haag, R. *Macromolecules* **2013**, *46*, 8458–8466.
65. Weiss, M. E. R.; Paulus, F.; Steinhilber, D.; Nikitin, A. N.; Haag, R.; Schütte, C. *Macromol. Theory Simul.* **2012**, *21*, 470–481.
66. Imran ul-haq, M.; Shenoi, R. A.; Brooks, D. E.; Kizhakkedathu, J. N. *J. Polym. Sci. Part A Polym. Chem.* **2013**, *51*, 2614–2621.
67. Kainthan, R. K.; Muliawan, E. B.; Hatzikiriakos, S. G.; Brooks, D. E. *Macromolecules* **2006**, *39*, 7708–7717.
68. Fitton, A. O.; Hill, J.; Jane, D. E.; Millar, R. *Synthesis* **1987**, *1987*, 1140–1142.
69. Hans, M.; Keul, H.; Moeller, M. *Polymer* **2009**, *50*, 1103–1108.
70. Taton, D.; Le Borgne, A.; Sepulchre, M.; Spassky, N. *Macromol. Chem. Phys.* **1994**, *195*, 139–148.
71. Erberich, M.; Keul, H.; Möller, M. *Macromolecules* **2007**, *40*, 3070–3079.

72. Gervais, M.; Brocas, A.-L.; Cendejas, G.; Deffieux, A.; Carlotti, S. *Macromolecules* **2010**, *43*, 1778–1784.
73. Sisson, A. L.; Steinhilber, D.; Rossow, T.; Welker, P.; Licha, K.; Haag, R. *Angew. Chem. Int. Ed.* **2009**, *48*, 7540–7545.
74. Hoogenboom, R. *Angew. Chem. Int. Ed.* **2009**, *48*, 7978–7994.
75. Luxenhofer, R.; Schulz, A.; Roques, C.; Li, S.; Bronich, T. K.; Batrakova, E. V.; Jordan, R.; Kabanov, A. V. *Biomaterials* **2010**, *31*, 4972–4979.
76. Guillerm, B.; Monge, S.; Lapinte, V.; Robin, J.-J. *Macromol. Rapid Commun.* **2012**, *33*, 1600–1612.
77. Kelly, A. M.; Wiesbrock, F. *Macromol. Rapid Commun.* **2012**, *33*, 1632–1647.
78. Farrugia, B. L.; Kempe, K.; Schubert, U. S.; Hoogenboom, R.; Dargaville, T. R. *Biomacromolecules* **2013**, *14*, 2724–2732.
79. Rueda, J. C.; Komber, H.; Cedrón, J. C.; Voit, B.; Shevtsova, G. *Macromol. Chem. Phys.* **2003**, *204*, 947–953.
80. Adams, N.; Schubert, U. S. *Adv. Drug Del. Rev.* **2007**, *59*, 1504–1520.
81. Woodle, M. C.; Engbers, C. M.; Zalipsky, S. *Bioconj. Chem.* **1994**, *5*, 493–496.
82. Gaertner, F. C.; Luxenhofer, R.; Blechert, B.; Jordan, R.; Essler, M. *J. Control. Release* **2007**, *119*, 291–300.
83. McGary, C. W. *J. Polym. Sci.* **1960**, *46*, 51–57.
84. Ulbricht, J.; Jordan, R.; Luxenhofer, R. *Biomaterials* **2014**, *35*, 4848–4861.
85. Sprecht, E. H.; Neuman, A.; Neher, H. T. *U.S. Pat. 2773063* **1956**.
86. Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163–249.
87. Naito, H.; Takewa, Y.; Mizuno, T.; Ohya, S.; Nakayama, Y.; Tatsumi, E.; Kitamura, S.; Takano, H.; Taniguchi, S.; Taenaka, Y. *ASAIO J.* **2004**, *50*, 344–348.
88. Ohya, S.; Nakayama, Y.; Matsuda, T. *Biomacromolecules* **2001**, *2*, 856–863.
89. Guan, Y.; Zhang, Y. *Soft Matter* **2011**, *7*, 6375–6384.
90. Klouda, L.; Mikos, A. G. *Eur. J. Pharm. Biopharm.* **2008**, *68*, 34–45.
91. Cooperstein, M.; Canavan, H. *Biointerphases* **2013**, *8*, 1–12.
92. Hashmi, B.; Zarzar, L. D.; Mammoto, T.; Mammoto, A.; Jiang, A.; Aizenberg, J.; Ingber, D. E. *Adv. Mater.* **2014**, *26*, 3253–3257.
93. Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283–289.
94. Fundueanu, G.; Constantin, M.; Ascenzi, P. *Int. J. Pharm.* **2009**, *379*, 9–17.
95. Ju, H. K.; Kim, S. Y.; Kim, S. J.; Lee, Y. M. *J. Appl. Polym. Sci.* **2002**, *83*, 1128–1139.
96. Seiffert, S.; Thiele, J.; Abate, A. R.; Weitz, D. A. *J. Am. Chem. Soc.* **2010**, *132*, 6606–6609.
97. Seiffert, S.; Romanowsky, M. B.; Weitz, D. A. *Langmuir* **2010**, *26*, 14842–14847.
98. Johnson, J. A.; Lewis, D. R.; Díaz, D. D.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 6564–6565.
99. Willcock, H.; O'Reilly, R. K. *Polym. Chem.* **2010**, *1*, 149–157.
100. Ooi, H. W.; Jack, K. S.; Peng, H.; Whittaker, A. K. *Polym. Chem.* **2013**, *4*, 4788–4800.
101. Ooi, H. W.; Jack, K. S.; Whittaker, A. K.; Peng, H. *J. Polym. Sci. Part A Polym. Chem.* **2013**, *51*, 4626–4636.
102. Papavasiliou, G.; Songprawat, P.; Pérez-Luna, V.; Hammes, E.; Morris, M.; Chiu, Y. C.; Brey, E. *Tissue Eng. Part C Methods* **2008**, *14*, 129–140.
103. Oudshoorn, M. H. M.; Penterman, R.; Rissmann, R.; Bouwstra, J. A.; Broer, D. J.; Hennink, W. E. *Langmuir* **2007**, *23*, 11819–11825.
104. Chan, V.; Zorlutuna, P.; Jeong, J. H.; Kong, H.; Bashir, R. *Lab Chip* **2010**, *10*, 2062–2070.
105. Jongpaiboonkit, L.; King, W. J.; Lyons, G. E.; Paguirigan, A. L.; Warrick, J. W.; Beebe, D. J.; Murphy, W. L. *Biomaterials* **2008**, *29*, 3346–3356.
106. Lin, C.-C.; Sawicki, S. M.; Metters, A. T. *Biomacromolecules* **2007**, *9*, 75–83.
107. Fedorovich, N. E.; Oudshoorn, M. H.; van Geemen, D.; Hennink, W. E.; Alblas, J.; Dhert, W. J. A. *Biomaterials* **2009**, *30*, 344–353.
108. Hang, H. C.; Yu, C.; Kato, D. L.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14846–14851.

109. Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998.
110. Sletten, E. M.; Bertozzi, C. R. *Acc. Chem. Res.* **2011**, *44*, 666–676.
111. Finn, M. G.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1231–1232.
112. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.
113. Huisgen, R. *Angew. Chem. Int. Ed. Engl.* **1963**, *2*, 565–598.
114. Clark, M.; Kiser, P. *Polym. Int.* **2009**, *58*, 1190–1195.
115. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.
116. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
117. Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2004**, *127*, 210–216.
118. Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 2210–2215.
119. Rodionov, V. O.; Presolski, S. I.; Díaz Díaz, D.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12705–12712.
120. Worrell, B. T.; Malik, J. A.; Fokin, V. V. *Science* **2013**, *340*, 457–460.
121. Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, *2006*, 51–68.
122. Fournier, D.; Hoogenboom, R.; Schubert, U. S. *Chem. Soc. Rev.* **2007**, *36*, 1369–1380.
123. Lutz, J.-F. *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025.
124. van Berkel, S. S.; Dirks, A. J.; Debets, M. F.; van Delft, F. L.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; Rutjes, F. P. J. T. *ChemBioChem* **2007**, *8*, 1504–1508.
125. Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278–308.
126. Hein, C.; Liu, X.-M.; Wang, D. *Pharm. Res.* **2008**, *25*, 2216–2230.
127. Malkoch, M.; Vestberg, R.; Gupta, N.; Mespouille, L.; Dubois, P.; Mason, A. F.; Hedrick, J. L.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Hawker, C. J. *Chem. Commun.* **2006**, 2774–2776.
128. Liu, S. Q.; Rachel Ee, P. L.; Ke, C. Y.; Hedrick, J. L.; Yang, Y. Y. *Biomaterials* **2009**, *30*, 1453–1461.
129. Bertozzi, C. R. *unpublished results*.
130. Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686–4687.
131. Wittig, G.; Krebs, A. *Chem. Ber.* **1961**, *94*, 3260–3275.
132. Meier, H.; Petersen, H.; Kolshorn, H. *Chem. Ber.* **1980**, *113*, 2398–2409.
133. Turner, R. B.; Jarrett, A. D.; Goebel, P.; Mallon, B. J. *J. Am. Chem. Soc.* **1973**, *95*, 790–792.
134. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046–15047.
135. Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793–16797.
136. Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006**, *1*, 644–648.
137. Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664–667.
138. DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. *Nat. Mater.* **2009**, *8*, 659–664.
139. Fairbanks, B. D.; Sims, E. A.; Anseth, K. S.; Bowman, C. N. *Macromolecules* **2010**, *43*, 4113–4119.
140. van Geel, R.; Pruijn, G. J. M.; van Delft, F. L.; Boelens, W. C. *Bioconj. Chem.* **2012**, *23*, 392–398.
141. Dommerholt, J.; Schmidt, S.; Temming, R.; Hendriks, L. J. A.; Rutjes, F. P. J. T.; van Hest, J. C. M.; Lefeber, D. J.; Friedl, P.; van Delft, F. L. *Angew. Chem. Int. Ed.* **2010**, *49*, 9422–9425.
142. Posner, T. *Ber. Dtsch. Chem. Ges.* **1905**, *38*, 646–657.
143. Hoyle, C. E.; Lee, T. Y.; Roper, T. *J. Polym. Sci. Part A Polym. Chem.* **2004**, *42*, 5301–5338.
144. Hoyle, C. E.; Bowman, C. N. *Angew. Chem. Int. Ed.* **2010**, *49*, 1540–1573.
145. Lowe, A. B. *Polym. Chem.* **2010**, *1*, 17–36.
146. Dondoni, A. *Angew. Chem. Int. Ed.* **2008**, *47*, 8995–8997.
147. Kade, M. J.; Burke, D. J.; Hawker, C. J. *J. Polym. Sci. Part A Polym. Chem.* **2010**, *48*, 743–750.
148. McNair, O. D.; Sparks, B. J.; Janisse, A. P.; Brent, D. P.; Patton, D. L.; Savin, D. A. *Macromolecules* **2013**, *46*, 5614–5621.

149. Feidenhans'l, N. A.; Lafleur, J. P.; Jensen, T. G.; Kutter, J. P. *Electrophoresis* **2014**, *35*, 282–288.
150. Çubuk, S.; Kahraman, M. V.; Yetimoğlu, E. K.; Kenan, S. *Anal. Chim. Acta* **2014**, *812*, 215–221.
151. Ware, T.; Simon, D.; Liu, C.; Musa, T.; Vasudevan, S.; Sloan, A.; Keefer, E. W.; Rennaker, R. L.; Voit, W. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2014**, *102*, 1–11.
152. Fu, Y.; Kao, W. J. *J. Biomed. Mater. Res. A* **2011**, *98A*, 201–211.
153. Elbert, D. L.; Pratt, A. B.; Lutolf, M. P.; Halstenberg, S.; Hubbell, J. A. *J. Control. Release* **2001**, *76*, 11–25.
154. Jin, R.; Moreira Teixeira, L. S.; Krouwels, A.; Dijkstra, P. J.; van Blitterswijk, C. A.; Karperien, M.; Feijen, J. *Acta Biomater.* **2010**, *6*, 1968–1977.
155. Park, Y.; Lutolf, M. P.; Hubbell, J. A.; Hunziker, E. B.; Wong, M. *Tissue Eng.* **2004**, *10*, 515–522.
156. Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007–2010.
157. Staudinger, H.; Meyer, J. *Helv. Chim. Acta* **1919**, *2*, 635–646.
158. Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org. Lett.* **2000**, *2*, 2141–2143.
159. Gattás-Asfura, K. M.; Stabler, C. L. *Biomacromolecules* **2009**, *10*, 3122–3129.
160. Gattás-Asfura, K. M.; Fraker, C. A.; Stabler, C. L. *J. Biomed. Mater. Res. A* **2011**, *99A*, 47–57.
161. Blackman, M. L.; Royzen, M.; Fox, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 13518–13519.
162. Devaraj, N. K.; Weissleder, R.; Hilderbrand, S. A. *Bioconj. Chem.* **2008**, *19*, 2297–2299.
163. Thalhammer, F.; Wallfahrer, U.; Sauer, J. *Tetrahedron Lett.* **1990**, *31*, 6851–6854.
164. Alge, D. L.; Azagarsamy, M. A.; Donohue, D. F.; Anseth, K. S. *Biomacromolecules* **2013**, *14*, 949–953.
165. Sletten, E. M.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2011**, *133*, 17570–17573.
166. Seiffert, S. *Macromol. Rapid Commun.* **2011**, *32*, 1600–1609.
167. Seiffert, S. *Angew. Chem. Int. Ed.* **2013**, *52*, 11462–11468.
168. Chang, T. M. S. *Science* **1964**, *146*, 524–525.
169. Malmsten, M., Microgels in Drug Delivery. In *Microgel Suspensions*, Wiley-VCH Verlag GmbH & Co. KGaA: 2011; pp 375–405.
170. Su, S.; Ali, M. M.; Filipe, C. D. M.; Li, Y.; Pelton, R. *Biomacromolecules* **2008**, *9*, 935–941.
171. Terashima, T., Polymer Microgels for Catalysis. In *Encyclopedia of Polymer Science and Technology*, John Wiley & Sons, Inc.: 2002.
172. Jiang, Y.; Chen, J.; Deng, C.; Suuronen, E. J.; Zhong, Z. *Biomaterials* **2014**, *35*, 4969–4985.
173. Khademhosseini, A.; Langer, R.; Borenstein, J.; Vacanti, J. P. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2480–2487.
174. Liu Tsang, V.; Bhatia, S. N. *Adv. Drug Del. Rev.* **2004**, *56*, 1635–1647.
175. Tibbitt, M. W.; Anseth, K. S. *Biotechnol. Bioeng.* **2009**, *103*, 655–663.
176. Du, Y.; Ghodousi, M.; Lo, E.; Vidula, M. K.; Emiroglu, O.; Khademhosseini, A. *Biotechnol. Bioeng.* **2010**, *105*, 655–662.
177. Brandl, F.; Sommer, F.; Goepferich, A. *Biomaterials* **2007**, *28*, 134–146.
178. Discher, D. E.; Mooney, D. J.; Zandstra, P. W. *Science* **2009**, *324*, 1673–1677.
179. Trappmann, B.; Gautrot, J. E.; Connelly, J. T.; Strange, D. G. T.; Li, Y.; Oyen, M. L.; Cohen Stuart, M. A.; Boehm, H.; Li, B.; Vogel, V.; Spatz, J. P.; Watt, F. M.; Huck, W. T. S. *Nat. Mater.* **2012**, *11*, 642–649.
180. Khademhosseini, A.; Langer, R. *Biomaterials* **2007**, *28*, 5087–5092.
181. Orive, G.; Hernandez, R. M.; Gascon, A. R.; Calafiore, R.; Chang, T. M. S.; Vos, P. D.; Hortelano, G.; Hunkeler, D.; Lacik, I.; Shapiro, A. M. J.; Pedraz, J. L. *Nat. Med.* **2003**, *9*, 104–107.
182. Chang, T. M. S. *Nat. Rev. Drug Discov.* **2005**, *4*, 221–235.
183. Qi, M.; Strand, B. L.; Mørch, Y.; Lacík, I.; Wang, Y.; Salehi, P.; Barbaro, B.; Gangemi, A.; Kuechle, J.; Romagnoli, T.; Hansen, M. A.; Rodriguez, L. A.; Benedetti, E.; Hunkeler, D.; Skjåk-Bræk, G.; Oberholzer, J. *Artif. Cells Blood Substit. Biotechnol.* **2008**, *36*, 403–420.
184. Elliott, R. B.; Escobar, L.; Calafiore, R.; Basta, G.; Garkavenko, O.; Vasconcellos, A.; Bambra, C. *Transplant. Proc.* **2005**, *37*, 466–469.
185. Qi, M. *Advances in Medicine* **2014**, *2014*, DOI: 10.1155/2014/429710.
186. Seiffert, S. *J. Polym. Sci. Part A Polym. Chem.* **2014**, *52*, 435–449.

187. Pich, A.; Richtering, W., Microgels by Precipitation Polymerization: Synthesis, Characterization, and Functionalization. In *Chemical Design of Responsive Microgels*, Pich, A.; Richtering, W., Eds. Springer Berlin Heidelberg: 2011; Vol. 234, pp 1–37.
188. Tumarkin, E.; Kumacheva, E. *Chem. Soc. Rev.* **2009**, *38*, 2161–2168.
189. Theberge, A. B.; Courtois, F.; Schaerli, Y.; Fischlechner, M.; Abell, C.; Hollfelder, F.; Huck, W. T. S. *Angew. Chem. Int. Ed.* **2010**, *49*, 5846–5868.
190. Wang, J.-T.; Wang, J.; Han, J.-J. *Small* **2011**, *7*, 1728–1754.
191. Dendukuri, D.; Doyle, P. S. *Adv. Mater.* **2009**, *21*, 4071–4086.
192. McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H.; Schueller, O. J. A.; Whitesides, G. M. *Electrophoresis* **2000**, *21*, 27–40.
193. Shah, R. K.; Shum, H. C.; Rowat, A. C.; Lee, D.; Agresti, J. J.; Utada, A. S.; Chu, L.-Y.; Kim, J.-W.; Fernandez-Nieves, A.; Martinez, C. J.; Weitz, D. A. *Mater. Today* **2008**, *11*, 18–27.
194. Panda, P.; Ali, S.; Lo, E.; Chung, B. G.; Hatton, T. A.; Khademhosseini, A.; Doyle, P. S. *Lab Chip* **2008**, *8*, 1056–1061.
195. Bott, K.; Upton, Z.; Schrobback, K.; Ehrbar, M.; Hubbell, J. A.; Lutolf, M. P.; Rizzi, S. C. *Biomaterials* **2010**, *31*, 8454–8464.
196. Yoshida, R.; Okano, T., Stimuli-Responsive Hydrogels and Their Application to Functional Materials. In *Biomedical Applications of Hydrogels Handbook*, Ottenbrite, R. M.; Park, K.; Okano, T., Eds. Springer New York: 2010; pp 19–43.
197. Gupta, P.; Vermani, K.; Garg, S. *Drug Discov. Today* **2002**, *7*, 569–579.
198. Zhao, Y.-L.; Stoddart, J. F. *Langmuir* **2009**, *25*, 8442–8446.
199. Shiga, T., Deformation and Viscoelastic Behavior of Polymer Gels in Electric Fields. In *Neutron Spin Echo Spectroscopy Viscoelasticity Rheology*, Springer Berlin Heidelberg: 1997; Vol. 134, pp 131–163.
200. Satarkar, N. S.; Zach Hilt, J. *Acta Biomater.* **2008**, *4*, 11–16.
201. Kurisawa, M.; Terano, M.; Yui, N. *J. Biomater. Sci. Polym. Ed.* **1997**, *8*, 691–708.
202. Huang, W. M.; Ding, Z.; Wang, C. C.; Wei, J.; Zhao, Y.; Purnawali, H. *Mater. Today* **2010**, *13*, 54–61.
203. 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.
204. Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *46*, 6387–6392.
205. Maeda, H.; Greish, K.; Fang, J., The EPR Effect and Polymeric Drugs: A Paradigm Shift for Cancer Chemotherapy in the 21st Century. In *Polymer Therapeutics II*, Satchi-Fainaro, R.; Duncan, R., Eds. Springer Berlin Heidelberg: 2006; Vol. 193, pp 103–121.
206. Mura, S.; Nicolas, J.; Couvreur, P. *Nat. Mater.* **2013**, *12*, 991–1003.
207. Khetan, S.; Guvendiren, M.; Legant, W. R.; Cohen, D. M.; Chen, C. S.; Burdick, J. A. *Nat. Mater.* **2013**, *12*, 458–465.
208. Lampe, K. J.; Bjugstad, K. B.; Mahoney, M. J. *Tissue Eng. Part A.* **2010**, *16*, 1857–1866.
209. Raeber, G. P.; Lutolf, M. P.; Hubbell, J. A. *Biophys. J.* **2005**, *89*, 1374–1388.
210. Cabane, E.; Zhang, X.; Langowska, K.; Palivan, C.; Meier, W. *Biointerphases* **2012**, *7*, 1–27.
211. Lutolf, M. P.; Raeber, G. P.; Zisch, A. H.; Tirelli, N.; Hubbell, J. A. *Adv. Mater.* **2003**, *15*, 888–892.
212. Hudalla, G. A.; Eng, T. S.; Murphy, W. L. *Biomacromolecules* **2008**, *9*, 842–849.
213. Kloxin, A. M.; Kasko, A. M.; Salinas, C. N.; Anseth, K. S. *Science* **2009**, *324*, 59–63.
214. Casey, J. R.; Grinstein, S.; Orlowski, J. *Nat. Rev. Mol. Cell Biol.* **2010**, *11*, 50–61.
215. Grotthuss, C. J. T. *Ann. Chim.* **1806**, *58*, 54–74.
216. Cukierman, S. *Biochim. Biophys. Acta* **2006**, *1757*, 876–885.
217. Santos, E.; Pedraz, J. L.; Hernández, R. M.; Orive, G. *J. Control. Release* **2013**, *170*, 1–14.
218. Parrott, M. C.; Luft, J. C.; Byrne, J. D.; Fain, J. H.; Napier, M. E.; DeSimone, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 17928–17932.
219. Murthy, N.; Xu, M.; Schuck, S.; Kunisawa, J.; Shastri, N.; Fréchet, J. M. J. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4995–5000.
220. Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 89–112.

221. Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1304–1319.
222. Lehn, J.-M. *Polym. Int.* **2002**, *51*, 825–839.
223. Wojtecki, R. J.; Meador, M. A.; Rowan, S. J. *Nat. Mater.* **2011**, *10*, 14–27.
224. Schneider, H.-J.; Yatsimirsky, A. K. *Chem. Soc. Rev.* **2008**, *37*, 263–277.
225. Lawrence, D. S.; Jiang, T.; Levett, M. *Chem. Rev.* **1995**, *95*, 2229–2260.
226. Martell, A. E.; Hancock, R. D.; Motekaitis, R. J. *Coord. Chem. Rev.* **1994**, *133*, 39–65.
227. Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681–1712.
228. Moore, J. S. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 108–116.
229. Aida, T.; Meijer, E. W.; Stupp, S. I. *Science* **2012**, *335*, 813–817.
230. Schubert, U. S.; Eschbaumer, C. *Angew. Chem. Int. Ed.* **2002**, *41*, 2892–2926.
231. Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071–4098.
232. 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.
233. Fox, J. D.; Rowan, S. J. *Macromolecules* **2009**, *42*, 6823–6835.
234. Sangeetha, N. M.; Maitra, U. *Chem. Soc. Rev.* **2005**, *34*, 821–836.
235. Dastidar, P. *Chem. Soc. Rev.* **2008**, *37*, 2699–2715.
236. Piepenbrock, M.-O. M.; Lloyd, G. O.; Clarke, N.; Steed, J. W. *Chem. Rev.* **2009**, *110*, 1960–2004.
237. Smith, D. K. *Nat. Chem.* **2010**, *2*, 162–163.
238. Noro, A.; Hayashi, M.; Matsushita, Y. *Soft Matter* **2012**, *8*, 6416–6429.
239. Seiffert, S.; Sprakel, J. *Chem. Soc. Rev.* **2012**, *41*, 909–930.
240. Binder, W. H.; Zirbs, R. *Adv. Polym. Sci.* **2007**, *207*, 1–78.
241. Yu, X.; Samanta, B.; Xu, H.; Arumugam, P.; Ofir, Y.; Jordan, B. J.; Rotello, V. M. *Small* **2009**, *5*, 86–89.
242. Boyd, A. S. F.; Carroll, J. B.; Cooke, G.; Garety, J. F.; Jordan, B. J.; Mabruk, S.; Rosair, G.; Rotello, V. M. *Chem. Commun.* **2005**, 2468–2470.
243. Whittell, G. R.; Hager, M. D.; Schubert, U. S.; Manners, I. *Nat. Mater.* **2011**, *10*, 176–188.
244. Lohmeijer, B. G. G.; Schubert, U. S. *J. Polym. Sci. Part A Polym. Chem.* **2003**, *41*, 1413–1427.
245. Tuncaboylu, D. C.; Sari, M.; Oppermann, W.; Okay, O. *Macromolecules* **2011**, *44*, 4997–5005.
246. Shao, H.; Parquette, J. R. *Chem. Commun.* **2010**, *46*, 4285–4287.
247. Burattini, S.; Colquhoun, H. M.; Fox, J. D.; Friedmann, D.; Greenland, B. W.; Harris, P. J. F.; Hayes, W.; Mackay, M. E.; Rowan, S. J. *Chem. Commun.* **2009**, 6717–6719.
248. Burattini, S.; Greenland, B. W.; Merino, D. H.; Weng, W.; Seppala, J.; Colquhoun, H. M.; Hayes, W.; Mackay, M. E.; Hamley, I. W.; Rowan, S. J. *J. Am. Chem. Soc.* **2010**, *132*, 12051–12058.
249. Varshey, D. B.; Sander, J. R. G.; Friščić, T.; MacGillivray, L. R., Supramolecular Interactions. In *Supramol. Chem.*, John Wiley & Sons, Ltd: 2012.
250. Grassi, G.; Farra, R.; Caliceti, P.; Guarnieri, G.; Salmaso, S.; Carenza, M.; Grassi, M. *Am. J. Drug Deliv.* **2005**, *3*, 239–251.
251. Lemmers, M.; Sprakel, J.; Voets, I. K.; van der Gucht, J.; Cohen Stuart, M. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 708–711.
252. Gao, L.; Zhang, Z.; Zheng, B.; Huang, F. *Polym. Chem.* **2014**.
253. Asoh, T.-A.; Yoshitake, H.; Takano, Y.; Kikuchi, A. *Macromol. Chem. Phys.* **2013**, *214*, 2534–2539.
254. Zhang, Y.; Zhang, W.; Li, J.; Dang, J.; Wei, T. *Mater. Lett.* **2012**, *82*, 227–229.
255. Qiu, Y.; Park, K. *Adv. Drug Del. Rev.* **2001**, *53*, 321–339.
256. Dankers, P. Y. W.; Meijer, E. W. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 2047–2073.
257. Cordier, P.; Tournilhac, F.; Soulie-Ziakovic, C.; Leibler, L. *Nature* **2008**, *451*, 977–980.
258. Murphy, E. B.; Wudl, F. *Prog. Polym. Sci.* **2010**, *35*, 223–251.
259. Li, J.; Viveros, J. A.; Wrue, M. H.; Anthamatten, M. *Adv. Mater.* **2007**, *19*, 2851–2855.
260. West, J. L.; Hubbell, J. A. *Biomaterials* **1995**, *16*, 1153–1156.

261. Osada, K.; Kataoka, K., Drug and Gene Delivery Based on Supramolecular Assembly of PEG-Polypeptide Hybrid Block Copolymers. In *Peptide Hybrid Polymers*, Klok, H.-A.; Schlaad, H., Eds. Springer Berlin Heidelberg: 2006; Vol. 202, pp 113–153.
262. Alves, M.-H.; Jensen, B. E. B.; Smith, A. A. A.; Zelikin, A. N. *Macromol. Biosci.* **2011**, *11*, 1293–1313.
263. Abdel-Mottaleb, M. M. A.; Mortada, N. D.; El-Shamy, A. A.; Awad, G. A. S. *Drug Dev. Ind. Pharm.* **2009**, *35*, 311–320.
264. Hassan, C.; Peppas, N., Structure and Applications of Poly(vinyl alcohol) Hydrogels Produced by Conventional Crosslinking or by Freezing/Thawing Methods. In *Biopolymers · PVA Hydrogels, Anionic Polymerisation Nanocomposites*, Springer Berlin Heidelberg: 2000; Vol. 153, pp 37–65.
265. Radowski, M. R.; Shukla, A.; von Berlepsch, H.; Böttcher, C.; Pickaert, G.; Rehage, H.; Haag, R. *Angew. Chem. Int. Ed.* **2007**, *46*, 1265–1269.
266. Zieringer, M.; Wyszogrodzka, M.; Biskup, K.; Haag, R. *New J. Chem.* **2012**, *36*, 402–406.
267. Merschky, M.; Wyszogrodzka, M.; Haag, R.; Schmuck, C. *Chem. Eur. J.* **2010**, *16*, 14242–14246.
268. Raz, N.; Li, J. K.; Fiddes, L. K.; Tumarkin, E.; Walker, G. C.; Kumacheva, E. *Macromolecules* **2010**, *43*, 7277–7281.
269. George, M.; Abraham, T. E. *J. Control. Release* **2006**, *114*, 1–14.
270. Smidsrød, O. *Trends Biotechnol.* **1990**, *8*, 71–78.
271. Zhang, H.; Tumarkin, E.; Peerani, R.; Nie, Z.; Sullan, R. M. A.; Walker, G. C.; Kumacheva, E. *J. Am. Chem. Soc.* **2006**, *128*, 12205–12210.
272. Ishida, K.; Kuroda, R.; Miwa, M.; Tabata, Y.; Hokugo, A.; Kawamoto, T.; Sasaki, K.; Doita, M.; Kurosaka, M. *Tissue Eng.* **2007**, *13*, 1103–1112.
273. Boucard, N.; Viton, C.; Agay, D.; Mari, E.; Roger, T.; Chancerelle, Y.; Domard, A. *Biomaterials* **2007**, *28*, 3478–3488.
274. Van Vlierberghe, S.; Dubruel, P.; Schacht, E. *Biomacromolecules* **2011**, *12*, 1387–1408.
275. Perez-Castillejos, R. *Mater. Today* **2010**, *13*, 32–41.
276. Fu, S.; Thacker, A.; Sperger, D.; Boni, R.; Velankar, S.; Munson, E.; Block, L. *AAPS Pharm. Sci. Tech.* **2011**, *12*, 449–449.
277. Paszek, M. J.; Zahir, N.; Johnson, K. R.; Lakins, J. N.; Rozenberg, G. I.; Gefen, A.; Reinhart-King, C. A.; Margulies, S. S.; Dembo, M.; Boettiger, D.; Hammer, D. A.; Weaver, V. M. *Cancer Cell* **2005**, *8*, 241–254.
278. Ota, T.; Gilbert, T. W.; Schwartzman, D.; McTiernan, C. F.; Kitajima, T.; Ito, Y.; Sawa, Y.; Badylak, S. F.; Zenati, M. A. *J. Thorac. Cardiovasc. Surg.* **2008**, *136*, 1309–1317.
279. Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem. Int. Ed.* **2001**, *40*, 2382–2426.
280. Armstrong, G.; Buggy, M. *J. Mater. Sci.* **2005**, *40*, 547–559.
281. Wilson, A. J. *Soft Matter* **2007**, *3*, 409–425.
282. Sherrington, D. C.; Taskinen, K. A. *Chem. Soc. Rev.* **2001**, *30*, 83–93.
283. Cooke, G.; Rotello, V. M. *Chem. Soc. Rev.* **2002**, *31*, 275–286.
284. Binder, W.; Zirbs, R., Supramolecular Polymers and Networkswith Hydrogen Bonds in the Main- and Side-Chain. In *Hydrogen Bonded Polymers*, Binder, W., Ed. Springer Berlin Heidelberg: 2007; Vol. 207, pp 1–78.
285. Nernst, W. *Z. Phys. Chem.* **1891**, *8*, 110–139.
286. Bernal, J. D.; Megaw, H. D. *Proc. R. Soc. London A* **1935**, *151*, 384–420.
287. Huggins, M. L. *J. Org. Chem.* **1936**, *01*, 407–456.
288. Murray, T. J.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1992**, *114*, 4010–4011.
289. Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008–2010.
290. Pranata, J.; Wierschke, S. G.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810–2819.
291. Sartorius, J.; Schneider, H.-J. *Chem. Eur. J.* **1996**, *2*, 1446–1452.
292. Stadler, R.; Lucca Freitas, L. *Colloid. Polym. Sci.* **1986**, *264*, 773–778.
293. De Lucca Freitas, L. L.; Stadler, R. *Macromolecules* **1987**, *20*, 2478–2485.
294. Lucca Freitas, L.; Stadler, R. *Colloid. Polym. Sci.* **1988**, *266*, 1095–1101.

295. Beijer, F. H.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 6761–6769.
296. Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601–1604.
297. Beijer, F. H.; Kooijman, H.; Spek, A. L.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem. Int. Ed.* **1998**, *37*, 75–78.
298. Cates, M. E. *Macromolecules* **1987**, *20*, 2289–2296.
299. Cates, M. E.; Candau, S. J. *J. Phys.: Condens. Matter* **1990**, *2*, 6869–6892.
300. Cates, M. E. *J. Phys. France* **1988**, *49*, 1593–1600.
301. Knoben, W.; Besseling, N. A. M.; Bouteiller, L.; Cohen Stuart, M. A. *Phys. Chem. Chem. Phys.* **2005**, *7*, 2390–2398.
302. Knoben, W.; Besseling, N. A. M.; Cohen Stuart, M. A. *J. Chem. Phys.* **2007**, *126*, 024907.
303. Vermonden, T.; van Steenbergen, M. J.; Besseling, N. A. M.; Marcelis, A. T. M.; Hennink, W. E.; Sudhölter, E. J. R.; Cohen Stuart, M. A. *J. Am. Chem. Soc.* **2004**, *126*, 15802–15808.
304. van der Gucht, J.; Besseling, N. A. M.; Knoben, W.; Bouteiller, L.; Cohen Stuart, M. A. *Phys. Rev. E* **2003**, *67*, 051106.
305. Sprakel, J.; van der Gucht, J.; Cohen Stuart, M. A.; Besseling, N. A. M. *Phys. Rev. E* **2008**, *77*, 061502.
306. Lange, R. F. M.; Van Gurp, M.; Meijer, E. W. *J. Polym. Sci. Part A Polym. Chem.* **1999**, *37*, 3657–3670.
307. 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.
308. Botterhuis, N. E.; van Beek, D. J. M.; van Gemert, G. M. L.; Bosman, A. W.; Sijbesma, R. P. *J. Polym. Sci. Part A Polym. Chem.* **2008**, *46*, 3877–3885.
309. 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.
310. Kautz, H.; van Beek, D. J. M.; Sijbesma, R. P.; Meijer, E. W. *Macromolecules* **2006**, *39*, 4265–4267.
311. Rieth, L. R.; Eaton, R. F.; Coates, G. W. *Angew. Chem. Int. Ed.* **2001**, *40*, 2153–2156.
312. Yamauchi, K.; Lizotte, J. R.; Long, T. E. *Macromolecules* **2003**, *36*, 1083–1088.
313. Elkins, C. L.; Park, T.; McKee, M. G.; Long, T. E. *J. Polym. Sci. Part A Polym. Chem.* **2005**, *43*, 4618–4631.
314. McKee, M. G.; Elkins, C. L.; Park, T.; Long, T. E. *Macromolecules* **2005**, *38*, 6015–6023.
315. Feldman, K. E.; Kade, M. J.; Meijer, E. W.; Hawker, C. J.; Kramer, E. J. *Macromolecules* **2009**, *42*, 9072–9081.
316. Sijbesma, R. P.; Meijer, E. W. *Chem. Commun.* **2003**, 5–16.
317. Park, T.; Zimmerman, S. C.; Nakashima, S. *J. Am. Chem. Soc.* **2005**, *127*, 6520–6521.
318. Park, T.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2006**, *128*, 14236–14237.
319. Park, T.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2006**, *128*, 11582–11590.
320. Wang, X.-Z.; Li, X.-Q.; Shao, X.-B.; Zhao, X.; Deng, P.; Jiang, X.-K.; Li, Z.-T.; Chen, Y.-Q. *Chem. Eur. J.* **2003**, *9*, 2904–2913.
321. Nair, K. P.; Breedveld, V.; Weck, M. *Macromolecules* **2008**, *41*, 3429–3438.
322. Herbst, F.; Schröter, K.; Gunkel, I.; Gröger, S.; Thurn-Albrecht, T.; Balbach, J.; Binder, W. H. *Macromolecules* **2010**, *43*, 10006–10016.
323. Wietor, J.-L.; van Beek, D. J. M.; Peters, G. W.; Mendes, E.; Sijbesma, R. P. *Macromolecules* **2011**, *44*, 1211–1219.
324. Sivakova, S.; Bohnsack, D. A.; Mackay, M. E.; Suwanmala, P.; Rowan, S. J. *J. Am. Chem. Soc.* **2005**, *127*, 18202–18211.
325. Colombani, O.; Barioz, C.; Bouteiller, L.; Chanéac, C.; Fompérie, L.; Lortie, F.; Montès, H. *Macromolecules* **2005**, *38*, 1752–1759.
326. Mark, J. E.; Andrady, A. L. *Rubber Chem. Technol.* **1981**, *54*, 366–373.
327. Di Lorenzo, F.; Seiffert, S. *Macromol. Chem. Phys.* **2014**, DOI: 10.1002/macp.201400317.
328. Nair, K. P.; Breedveld, V.; Weck, M. *Soft Matter* **2011**, *7*, 553–559.

329. Ilhan, F.; Galow, T. H.; Gray, M.; Clavier, G.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 5895–5896.
330. Uzun, O.; Sanyal, A.; Nakade, H.; Thibault, R. J.; Rotello, V. M. *J. Am. Chem. Soc.* **2004**, *126*, 14773–14777.
331. Thibault, R. J.; Galow, T. H.; Turnberg, E. J.; Gray, M.; Hotchkiss, P. J.; Rotello, V. M. *J. Am. Chem. Soc.* **2002**, *124*, 15249–15254.
332. Thibault, R. J.; Hotchkiss, P. J.; Gray, M.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 11249–11252.
333. Drechsler, U.; Thibault, R. J.; Rotello, V. M. *Macromolecules* **2002**, *35*, 9621–9623.
334. Noro, A.; Matsushita, Y.; Lodge, T. P. *Macromolecules* **2009**, *42*, 5802–5810.
335. Fages, F. *Angew. Chem. Int. Ed.* **2006**, *45*, 1680–1682.
336. Brassinne, J.; Fustin, C.-A.; Gohy, J.-F. *J. Inorg. Organomet. Polym. Mater.* **2013**, *23*, 24–40.
337. Hofmeier, H.; Schubert, U. S. *Chem. Soc. Rev.* **2004**, *33*, 373–399.
338. Hofmeier, H.; Schubert, U. S. *Macromol. Chem. Phys.* **2003**, *204*, 1391–1397.
339. Calzia, K. J.; Tew, G. N. *Macromolecules* **2002**, *35*, 6090–6093.
340. Meier, M. A. R.; Schubert, U. S. *J. Polym. Sci. Part A Polym. Chem.* **2003**, *41*, 2964–2973.
341. Ott, C.; Ulbricht, C.; Hoogenboom, R.; Schubert, U. S. *Macromol. Rapid Commun.* **2012**, *33*, 556–561.
342. Schmatloch, S.; Schubert, U. S. *Macromol. Symp.* **2003**, *199*, 483–498.
343. El-ghayoury, A.; Hofmeier, H.; de Ruiter, B.; Schubert, U. S. *Macromolecules* **2003**, *36*, 3955–3959.
344. Kokil, A.; Yao, P.; Weder, C. *Macromolecules* **2005**, *38*, 3800–3807.
345. Meudtner, R. M.; Hecht, S. *Macromol. Rapid Commun.* **2008**, *29*, 347–351.
346. Meudtner, R. M.; Ostermeier, M.; Goddard, R.; Limberg, C.; Hecht, S. *Chem. Eur. J.* **2007**, *13*, 9834–9840.
347. Yuan, J.; Fang, X.; Zhang, L.; Hong, G.; Lin, Y.; Zheng, Q.; Xu, Y.; Ruan, Y.; Weng, W.; Xia, H.; Chen, G. *J. Mater. Chem.* **2012**, *22*, 11515–11522.
348. Beck, J. B.; Rowan, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 13922–13923.
349. Zhao, Y.; Beck, J. B.; Rowan, S. J.; Jamieson, A. M. *Macromolecules* **2004**, *37*, 3529–3531.
350. Rowan, S. J.; Beck, J. B. *Faraday Discuss.* **2005**, *128*, 43–53.
351. Weng, W.; Beck, J. B.; Jamieson, A. M.; Rowan, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 11663–11672.
352. Weng, W.; Jamieson, A. M.; Rowan, S. J. *Tetrahedron* **2007**, *63*, 7419–7431.
353. Weng, W.; Li, Z.; Jamieson, A. M.; Rowan, S. J. *Macromolecules* **2008**, *42*, 236–246.
354. Weng, W.; Li, Z.; Jamieson, A. M.; Rowan, S. J. *Soft Matter* **2009**, *5*, 4647–4657.
355. Pollino, J. M.; Nair, K. P.; Stubbs, L. P.; Adams, J.; Weck, M. *Tetrahedron* **2004**, *60*, 7205–7215.
356. Nair, K. P.; Breedveld, V.; Weck, M. *Macromolecules* **2011**, *44*, 3346–3357.
357. Yount, W. C.; Loveless, D. M.; Craig, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 14488–14496.
358. Yount, W. C.; Loveless, D. M.; Craig, S. L. *Angew. Chem. Int. Ed.* **2005**, *44*, 2746–2748.
359. Noro, A.; Matsushima, S.; He, X.; Hayashi, M.; Matsushita, Y. *Macromolecules* **2013**, *46*, 8304–8310.
360. Kumpfer, J. R.; Wie, J. J.; Swanson, J. P.; Beyer, F. L.; Mackay, M. E.; Rowan, S. J. *Macromolecules* **2011**, *45*, 473–480.
361. Serpe, M. J.; Craig, S. L. *Langmuir* **2007**, *23*, 1626–1634.
362. Yount, W. C.; Juwarker, H.; Craig, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 15302–15303.
363. Loveless, D. M.; Jeon, S. L.; Craig, S. L. *Macromolecules* **2005**, *38*, 10171–10177.
364. Spruijt, E.; Sprakel, J.; Lemmers, M.; Stuart, M. A. C.; van der Gucht, J. *Phys. Rev. Lett.* **2010**, *105*, 208301.
365. Hofmeier, H.; Hoogenboom, R.; Wouters, M. E. L.; Schubert, U. S. *J. Am. Chem. Soc.* **2005**, *127*, 2913–2921.
366. Appel, E. A.; Biedermann, F.; Rauwald, U.; Jones, S. T.; Zayed, J. M.; Scherman, O. A. *J. Am. Chem. Soc.* **2010**, *132*, 14251–14260.
367. Lewis, C. L.; Stewart, K.; Anthamatten, M. *Macromolecules* **2014**, *47*, 729–740.

368. Paulusse, J. M. J.; van Beek, D. J. M.; Sijbesma, R. P. *J. Am. Chem. Soc.* **2007**, *129*, 2392–2397.
369. 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.
370. 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.
371. 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.
372. Song, G.; Zhang, L.; He, C.; Fang, D.-C.; Whitten, P. G.; Wang, H. *Macromolecules* **2013**, *46*, 7423–7435.
373. Klemm, D.; Heublein, B.; Fink, H. P.; Bohn, A. *Angew. Chem. Int. Ed.* **2005**, *44*, 3358–3393.
374. Chang, C.; Zhang, L. *Carbohydr. Polym.* **2011**, *84*, 40–53.
375. Xiao, C. *Starch - Stärke* **2013**, *65*, 82–88.
376. Fernández, E.; López, D.; Mijangos, C.; Duskova-Smrckova, M.; Ilavsky, M.; Dusek, K. *J. Polym. Sci. Part B Polym. Phys.* **2008**, *46*, 322–328.
377. Fernández, E.; Hernández, R.; Teresa Cuberes, M.; Mijangos, C.; López, D. *J. Polym. Sci. Part B Polym. Phys.* **2010**, *48*, 2403–2412.
378. Van Tomme, S. R.; Hennink, W. E. *Expert Rev. Med. Devices* **2007**, *4*, 147–164.
379. de Jong, S. J.; van Eerdenbrugh, B.; van Nostrum, C. F.; Kettenes-van den Bosch, J. J.; Hennink, W. E. *J. Control. Release* **2001**, *71*, 261–275.
380. 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.
381. Li, L.; Thangamathesvaran, P. M.; Yue, C. Y.; Tam, K. C.; Hu, X.; Lam, Y. C. *Langmuir* **2001**, *17*, 8062–8068.
382. Chang, C.; Lue, A.; Zhang, L. *Macromol. Chem. Phys.* **2008**, *209*, 1266–1273.
383. Dave, V.; Tamagno, M.; Focher, B.; Marsano, E. *Macromolecules* **1995**, *28*, 3531–3539.
384. Takegawa, A.; Murakami, M.-a.; Kaneko, Y.; Kadokawa, J.-i. *Carbohydr. Polym.* **2010**, *79*, 85–90.
385. Liu, Z.; Wang, H.; Li, B.; Liu, C.; Jiang, Y.; Yu, G.; Mu, X. *J. Mater. Chem.* **2012**, *22*, 15085–15091.
386. Pourjavadi, A.; Barzegar, S.; Mahdavinia, G. R. *Carbohydr. Polym.* **2006**, *66*, 386–395.
387. Gupta, D.; Tator, C. H.; Shoichet, M. S. *Biomaterials* **2006**, *27*, 2370–2379.
388. 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.
389. Wang, Y.; Lapitsky, Y.; Kang, C. E.; Shoichet, M. S. *J. Control. Release* **2009**, *140*, 218–223.
390. Fiore, G. L.; Klinkenberg, J. L.; Pfister, A.; Fraser, C. L. *Biomacromolecules* **2008**, *10*, 128–133.
391. Lee, H.; Dellatore, S. M.; Miller, W. M.; Messersmith, P. B. *Science* **2007**, *318*, 426–430.
392. Lee, H.; Scherer, N. F.; Messersmith, P. B. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12999–13003.
393. 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. USA* **2011**, *108*, 2651–2655.
394. Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 5362–5370.
395. Menyo, M. S.; Hawker, C. J.; Waite, J. H. *Soft Matter* **2013**, *9*, 10314–10323.
396. Peng, F.; Li, G.; Liu, X.; Wu, S.; Tong, Z. *J. Am. Chem. Soc.* **2008**, *130*, 16166–16167.
397. Spychaj, T.; Schmidt, B. *Macromol. Symp.* **2000**, *152*, 173–189.
398. Augst, A. D.; Kong, H. J.; Mooney, D. J. *Macromol. Biosci.* **2006**, *6*, 623–633.
399. Lee, K. Y.; Rowley, J. A.; Eiselt, P.; Moy, E. M.; Bouhadir, K. H.; Mooney, D. J. *Macromolecules* **2000**, *33*, 4291–4294.
400. Shapiro, L.; Cohen, S. *Biomaterials* **1997**, *18*, 583–590.
401. Liew, C. V.; Chan, L. W.; Ching, A. L.; Heng, P. W. S. *Int. J. Pharm.* **2006**, *309*, 25–37.
402. Hashimoto, T.; Suzuki, Y.; Tanihara, M.; Kakimaru, Y.; Suzuki, K. *Biomaterials* **2004**, *25*, 1407–1414.

403. Rowley, J. A.; Madlambayan, G.; Mooney, D. J. *Biomaterials* **1999**, *20*, 45–53.
404. Nunamaker, E. A.; Purcell, E. K.; Kipke, D. R. *J. Biomed. Mater. Res. A* **2007**, *83A*, 1128–1137.
405. Boontheekul, T.; Kong, H.-J.; Mooney, D. J. *Biomaterials* **2005**, *26*, 2455–2465.
406. Shachar, M.; Tsur-Gang, O.; Dvir, T.; Leor, J.; Cohen, S. *Acta Biomater.* **2011**, *7*, 152–162.
407. Kang, S.-W.; Cha, B.-H.; Park, H.; Park, K.-S.; Lee, K. Y.; Lee, S.-H. *Macromol. Biosci.* **2011**, *11*, 673–679.
408. Bidarra, S. J.; Barrias, C. C.; Fonseca, K. B.; Barbosa, M. A.; Soares, R. A.; Granja, P. L. *Biomaterials* **2011**, *32*, 7897–7904.
409. Novikova, L. N.; Mosahebi, A.; Wiberg, M.; Terenghi, G.; Kellerth, J.-O.; Novikov, L. N. *J. Biomed. Mater. Res. A* **2006**, *77A*, 242–252.
410. Kim, G.; Ahn, S.; Kim, Y.; Cho, Y.; Chun, W. *J. Mater. Chem.* **2011**, *21*, 6165–6172.
411. Appel, E. A.; del Barrio, J.; Loh, X. J.; Scherman, O. A. *Chem. Soc. Rev.* **2012**, *41*, 6195–6214.
412. Steed, J. W.; Atwood, J. L., Supramolecular Polymers, Gels and Fibres. In *Supramol. Chem.*, John Wiley & Sons, Ltd: 2009; pp 861–897.
413. Zheng, B.; Wang, F.; Dong, S.; Huang, F. *Chem. Soc. Rev.* **2012**, *41*, 1621–1636.
414. Gokel, G. W.; Leevy, W. M.; Weber, M. E. *Chem. Rev.* **2004**, *104*, 2723–2750.
415. Drain, C. M.; Varotto, A.; Radivojevic, I. *Chem. Rev.* **2009**, *109*, 1630–1658.
416. Li, W.-S.; Aida, T. *Chem. Rev.* **2009**, *109*, 6047–6076.
417. Ramaiah, D.; Neelakandan, P. P.; Nair, A. K.; Avirah, R. R. *Chem. Soc. Rev.* **2010**, *39*, 4158–4168.
418. Evans, N. H.; Beer, P. D. *Chem. Soc. Rev.* **2014**, *43*, 4658–4683.
419. Sliwa, W.; Deska, M. *Chem. Heterocycl. Compd.* **2002**, *38*, 646–667.
420. Hooley, R. J.; Rebek Jr, J. *Chem. Biol.* **2009**, *16*, 255–264.
421. Hardie, M. J., Cyclotriveratrylene and Cryptophanes. In *Supramol. Chem.*, John Wiley & Sons, Ltd: 2012.
422. Nimse, S. B.; Kim, T. *Chem. Soc. Rev.* **2013**, *42*, 366–386.
423. Warmuth, R., Carcerands and Hemicarcerands. In *Supramol. Chem.*, John Wiley & Sons, Ltd: 2012.
424. Li, J., Cyclodextrin Inclusion Polymers Forming Hydrogels. In *Inclusion Polymers*, Wenz, G., Ed. Springer Berlin Heidelberg: 2009; Vol. 222, pp 175–203.
425. Chen, G.; Jiang, M. *Chem. Soc. Rev.* **2011**, *40*, 2254–2266.
426. Harada, A.; Takashima, Y.; Yamaguchi, H. *Chem. Soc. Rev.* **2009**, *38*, 875–882.
427. Chen, Y.; Liu, Y. *Chem. Soc. Rev.* **2010**, *39*, 495–505.
428. Kim, K.; Selvapalam, N.; Ko, Y. H.; Park, K. M.; Kim, D.; Kim, J. *Chem. Soc. Rev.* **2007**, *36*, 267–279.
429. Ni, X.-L.; Xiao, X.; Cong, H.; Liang, L.-L.; Cheng, K.; Cheng, X.-J.; Ji, N.-N.; Zhu, Q.-J.; Xue, S.-F.; Tao, Z. *Chem. Soc. Rev.* **2013**, *42*, 9480–9508.
430. Liu, Y.; Yang, H.; Wang, Z.; Zhang, X. *Chem. Asian J.* **2013**, *8*, 1626–1632.
431. Nakahata, M.; Takashima, Y.; Yamaguchi, H.; Harada, A. *Nat. Commun.* **2011**, *2*, 511.
432. Liu, S.; Ruspic, C.; Mukhopadhyay, P.; Chakrabarti, S.; Zavalij, P. Y.; Isaacs, L. *J. Am. Chem. Soc.* **2005**, *127*, 15959–15967.
433. Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem. Int. Ed.* **2005**, *44*, 4844–4870.
434. GRAS Notice No. GRN 000046, gamma-cyclodextrin, 2000.
435. Li, J.; Harada, A.; Kamachi, M. *Polym. J.* **1994**, *26*, 1019–1026.
436. Li, J. *NPG Asia Mater.* **2010**, *2*, 112–118.
437. Liu, K. L.; Zhang, Z.; Li, J. *Soft Matter* **2011**, *7*, 11290–11297.
438. Li, J.; Loh, X. J. *Adv. Drug Del. Rev.* **2008**, *60*, 1000–1017.
439. Joung, Y. K.; Ooya, T.; Yamaguchi, M.; Yui, N. *Adv. Mater.* **2007**, *19*, 396–400.
440. Kretschmann, O.; Choi, S. W.; Miyauchi, M.; Tomatsu, I.; Harada, A.; Ritter, H. *Angew. Chem. Int. Ed.* **2006**, *45*, 4361–4365.
441. Hetzer, M.; Schmidt, B. V. K. J.; Barner-Kowollik, C.; Ritter, H. *Polym. Chem.* **2014**, *5*, 2142–2152.

442. Charlot, A.; Auzély-Velty, R. *Macromolecules* **2007**, *40*, 9555–9563.
443. Koopmans, C.; Ritter, H. *Macromolecules* **2008**, *41*, 7418–7422.
444. Hwang, J.; Rodgers, K.; Oliver, J. C.; Schluep, T. *Int. J. Nanomed.* **2008**, *3*, 359–371.
445. Davis, M. E.; Brewster, M. E. *Nat. Rev. Drug Discov.* **2004**, *3*, 1023–1035.
446. Lee, M. S.; Kim, J.-C. *Polym. Int.* **2014**, *63*, 989–996.
447. Tomatsu, I.; Hashidzume, A.; Harada, A. *Macromol. Rapid Commun.* **2006**, *27*, 238–241.
448. Peng, K.; Tomatsu, I.; Kros, A. *Chem. Commun.* **2010**, *46*, 4094–4096.
449. Tamesue, S.; Takashima, Y.; Yamaguchi, H.; Shinkai, S.; Harada, A. *Angew. Chem. Int. Ed.* **2010**, *49*, 7461–7464.
450. Wang, Q.; Mynar, J. L.; Yoshida, M.; Lee, E.; Lee, M.; Okuro, K.; Kinbara, K.; Aida, T. *Nature* **2010**, *463*, 339–343.
451. 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.
452. Appel, E. A.; Loh, X. J.; Jones, S. T.; Biedermann, F.; Dreiss, C. A.; Scherman, O. A. *J. Am. Chem. Soc.* **2012**, *134*, 11767–11773.
453. Appel, E. A.; Loh, X. J.; Jones, S. T.; Dreiss, C. A.; Scherman, O. A. *Biomaterials* **2012**, *33*, 4646–4652.
454. Park, K. M.; Yang, J.-A.; Jung, H.; Yeom, J.; Park, J. S.; Park, K.-H.; Hoffman, A. S.; Hahn, S. K.; Kim, K. *ACS Nano* **2012**, *6*, 2960–2968.
455. Berger, J.; Reist, M.; Mayer, J.; Felt, O.; Peppas, N.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 19–34.
456. Berger, J.; Reist, M.; Mayer, J.; Felt, O.; Gurny, R. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 35–52.
457. Cohen Stuart, M. A.; Hofs, B.; Voets, I. K.; de Keizer, A. *Curr. Opin. Colloid Interface Sci.* **2005**, *10*, 30–36.
458. 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.
459. Krogstad, D. V.; Lynd, N. A.; Choi, S.-H.; Spruell, J. M.; Hawker, C. J.; Kramer, E. J.; Tirrell, M. V. *Macromolecules* **2013**, *46*, 1512–1518.
460. Giri, T. K.; Thakur, A.; Alexander, A.; Ajazuddin; Badwaik, H.; Tripathi, D. K. *Acta Pharm. Sin. B* **2012**, *2*, 439–449.
461. Sashiwa, H.; Aiba, S.-i. *Prog. Polym. Sci.* **2004**, *29*, 887–908.
462. Bhattarai, N.; Gunn, J.; Zhang, M. *Adv. Drug Del. Rev.* **2010**, *62*, 83–99.
463. Anchisi, C.; Meloni, M. C.; Maccioni, A. M. *Int. J. Cosmetic Sci.* **2007**, *29*, 485–485.
464. Dumitriu, S.; Magny, P.; Montane, D.; Vidal, P.; Chornet, E. *J. Bioact. Compat. Polym.* **1994**, *9*, 184–209.
465. Tan, H.; Chu, C. R.; Payne, K. A.; Marra, K. G. *Biomaterials* **2009**, *30*, 2499–2506.
466. Dai, Y.-N.; Li, P.; Zhang, J.-P.; Wang, A.-Q.; Wei, Q. *Biopharm. Drug Dispos.* **2008**, *29*, 173–184.
467. Reis, L. A.; Chiu, L. L. Y.; Liang, Y.; Hyunh, K.; Momen, A.; Radisic, M. *Acta Biomater.* **2012**, *8*, 1022–1036.
468. Harvestine, J. N.; Mikulski, B. A.; Mahuta, K. M.; Crouse, J. Z.; Guo, X.; Lee, J. C.; Midelfort, K. S.; Chen, J.; Zhang, W. *Part. Part. Syst. Charact.* **2014**, DOI: 10.1002/ppsc.201400002.
469. Jătariu, A. N.; Danu, M.; Peptu, C. A.; Ioanid, G.; Ibanescu, C.; Popa, M. *Soft Materials* **2011**, *11*, 45–54.
470. Magnin, D.; Lefebvre, J.; Chornet, E.; Dumitriu, S. *Carbohydr. Polym.* **2004**, *55*, 437–453.
471. Dumitriu, S.; Chornet, E. *Biotechnol. Prog.* **1997**, *13*, 539–545.
472. 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.
473. Shibayama, M.; Tanaka, T., Volume phase transition and related phenomena of polymer gels. In *Responsive Gels: Volume Transitions I*, Dušek, K., Ed. Springer Berlin Heidelberg: 1993; Vol. 109, pp 1–62.
474. Abdurrahmanoglu, S.; Can, V.; Okay, O. *Polymer* **2009**, *50*, 5449–5455.
475. Abdurrahmanoglu, S.; Cilingir, M.; Okay, O. *Polymer* **2011**, *52*, 694–699.

476. Tuncaboylu, D. C.; Sahin, M.; Argun, A.; Oppermann, W.; Okay, O. *Macromolecules* **2012**, *45*, 1991–2000.
477. Tuncaboylu, D. C.; Argun, A.; Sahin, M.; Sari, M.; Okay, O. *Polymer* **2012**, *53*, 5513–5522.
478. Argun, A.; Algi, M.; Tuncaboylu, D.; Okay, O. *Colloid. Polym. Sci.* **2014**, *292*, 511–517.
479. Tian, J.; Seery, T. A. P.; Weiss, R. A. *Macromolecules* **2004**, *37*, 9994–10000.
480. Tian, J.; Seery, T. A. P.; Ho, D. L.; Weiss, R. A. *Macromolecules* **2004**, *37*, 10001–10008.
481. Hao, J.; Weiss, R. A. *Macromolecules* **2011**, *44*, 9390–9398.
482. Hart, L. R.; Harries, J. L.; Greenland, B. W.; Colquhoun, H. M.; Hayes, W. *Polym. Chem.* **2013**, *4*, 4860–4870.
483. Burnworth, M.; Tang, L.; Kumpfer, J. R.; Duncan, A. J.; Beyer, F. L.; Fiore, G. L.; Rowan, S. J.; Weder, C. *Nature* **2011**, *472*, 334–337.
484. 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.
485. Bode, S.; Bose, R. K.; Matthes, S.; Ehrhardt, M.; Seifert, A.; Schacher, F. H.; Paulus, R. M.; Stumpf, S.; Sandmann, B.; Vitz, J.; Winter, A.; Hoeppener, S.; Garcia, S. J.; Spange, S.; van der Zwaag, S.; Hager, M. D.; Schubert, U. S. *Polym. Chem.* **2013**, *4*, 4966–4973.
486. Burattini, S.; Colquhoun, H. M.; Greenland, B. W.; Hayes, W. *Faraday Discuss.* **2009**, *143*, 251–264.
487. Greenland, B. W.; Burattini, S.; Hayes, W.; Colquhoun, H. M. *Tetrahedron* **2008**, *64*, 8346–8354.
488. Kumpfer, J. R.; Rowan, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 12866–12874.
489. Chen, S.; Cao, Q.; Jing, B.; Cai, Y.; Liu, P.; Hu, J. *J. Appl. Polym. Sci.* **2006**, *102*, 5224–5231.
490. Chen, S.; Hu, J.; Liu, Y.; Liem, H.; Zhu, Y.; Liu, Y. *J. Polym. Sci. Part B Polym. Phys.* **2007**, *45*, 444–454.
491. Chen, S.; Hu, J.; Zhuo, H.; Yuen, C.; Chan, L. *Polymer* **2010**, *51*, 240–248.
492. Tønnesen, H. H.; Karlsen, J. *Drug Dev. Ind. Pharm.* **2002**, *28*, 621–630.
493. Haag, R. *Angew. Chem. Int. Ed.* **2004**, *43*, 278–282.
494. Tao, C.-a.; Wang, J.; Qin, S.; Lv, Y.; Long, Y.; Zhu, H.; Jiang, Z. *J. Mater. Chem.* **2012**, *22*, 24856–24861.
495. 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. Healthcare Mater.* **2014**, *3*, 70–78.
496. Ma, D.; Tu, K.; Zhang, L.-M. *Biomacromolecules* **2010**, *11*, 2204–2212.
497. Gübeli, R. J.; Hövermann, D.; Seitz, H.; Rebmann, B.; Schoenmakers, R. G.; Ehrbar, M.; Charpin-El Hamri, G.; Daoud-El Baba, M.; Werner, M.; Müller, M.; Weber, W. *Adv. Funct. Mater.* **2013**, *23*, 5355–5362.
498. Tan, W. H.; Takeuchi, S. *Adv. Mater.* **2007**, *19*, 2696–2701.
499. Tumarkin, E.; Tzadu, L.; Csaszar, E.; Seo, M.; Zhang, H.; Lee, A.; Peerani, R.; Purpura, K.; Zandstra, P. W.; Kumacheva, E. *Integr. Biol.* **2011**, *3*, 653–662.
500. Kumachev, A.; Greener, J.; Tumarkin, E.; Eiser, E.; Zandstra, P. W.; Kumacheva, E. *Biomaterials* **2011**, *32*, 1477–1483.
501. Sakai, S.; Ito, S.; Inagaki, H.; Hirose, K.; Matsuyama, T.; Taya, M.; Kawakami, K. *Biomicrofluidics* **2011**, *5*, 013402.
502. Martinez, C. J.; Kim, J. W.; Ye, C.; Ortiz, I.; Rowat, A. C.; Marquez, M.; Weitz, D. *Macromol. Biosci.* **2012**, *12*, 946–951.
503. Sugiura, S.; Oda, T.; Izumida, Y.; Aoyagi, Y.; Satake, M.; Ochiai, A.; Ohkohchi, N.; Nakajima, M. *Biomaterials* **2005**, *26*, 3327–3331.
504. Velasco, D.; Chau, M.; Therien-Aubin, H.; Kumachev, A.; Tumarkin, E.; Jia, Z.; Walker, G. C.; Monteiro, M. J.; Kumacheva, E. *Soft Matter* **2013**, *9*, 2380–2383.

# 7 Publications and Conference Contributions

## Publications with Peer Review Process

[1] T. Rossow, J. A. Heyman, A. J. Ehrlicher, A. Langhoff, D. A. Weitz, R. Haag, and S. Seiffert, *J. Am. Chem. Soc.* **2012**, *134*, 4983–4989, *Controlled Synthesis of Cell-Laden Microgels by Radical-Free Gelation in Droplet Microfluidics.*

[2] T. Rossow, S. Hackelbusch, P. Van Assenbergh, and S. Seiffert, *Polym. Chem.* **2013**, *4*, 2515–2527, *A Modular Construction Kit for Supramolecular Polymer Gels.*

[3] T. Rossow, S. Bayer, R. Albrecht, C. C. Tzschucke, and S. Seiffert, *Macromol. Rapid Commun.* **2013**, *34*, 1401–1407, *Supramolecular Hydrogel Capsules Based on PEG: A Step Toward Degradable Biomaterials with Rational Design.*

[4] S. Hackelbusch, T. Rossow, P. Van Assenbergh, and S. Seiffert, *Macromolecules* **2013**, *46*, 6273–6286, *Chain Dynamics in Supramolecular Polymer Networks.*

[5] D. Steinhilber,* T. Rossow,* S. Wedepohl, F. Paulus, S. Seiffert, and R. Haag, *Angew. Chem. Int. Ed.* **2013**, *52*, 13538–13543; *Angew. Chem.* **2013**, *125*, 13780–13785, *A Microgel Construction Kit for Bioorthogonal Encapsulation and pH-Controlled Release of Living Cells.* [*equal contribution]

[6] T. Rossow and S. Seiffert, *Polym. Chem.* **2014**, *5*, 3018–3029, *Supramolecular Polymer Gels with Potential Model-Network Structure.*

[7] S. Hackelbusch,* T. Rossow,* H. Becker, and S. Seiffert, *Macromolecules* **2014**, *47*, 4028–4036, *Multiresponsive Polymer Hydrogels by Orthogonal Supramolecular Chain Crosslinking.* [*equal contribution]

[8] D. Hövermann,* T. Rossow,* R. J. Gübeli, S. Seiffert, and W. Weber, *Macromol. Biosci.* **2014**, DOI: 10.1002/mabi.201400342, *Microfluidic Synthesis of Pharmacologically Responsive Supramolecular Biohybrid Microgels.* [*equal contribution]

## Publications without Peer Review Process

[9] D. Steinhilber, I. Papp, F. Paulus, T. Rossow, S. Seiffert, and R. Haag, *Macromol. Chem. Phys.* **2013**, *214,* F97–F98, *Multifunctional Dendritic Polyglycerol Nano- and Microgels by Miniemulsion, Nanoprecipitation and Microfluidic Techniques.*

## Conference Contributions

### Oral Presentations

[1] T. Rossow, R. Haag, S. Seiffert
"Polymer Synthesis and Biology with Droplet-Based Microfluidic Devices" *Bilateral workshop of the Freie Universität Berlin and the University of British Columbia*, Vancouver, Canada, 2012.

[2] T. Rossow, S. Seiffert
"Controlled Synthesis of Cell-Laden Degradable Microgels by Radical-Free and Supramolecular Gelation in Droplet Microfluidics" *15th JCF Spring Symposium of the GDCh,* Berlin, Germany, 2013.

[3] T. Rossow, D. Steinhilber, S. Seiffert, R. Haag
"A Microgel Construction Kit for Bioorthogonal Encapsulation and pH-Controlled Release of Living Cells" *4th BSRT Symposium: Regeneration is Communication,* Berlin, Germany, 2013.

[4] T. Rossow, D. Steinhilber, R. Haag, S. Seiffert
"Stimuli Responsive Cell-Laden Microgels by Click- and Supramolecular Chemistry in Droplet Microfluidics" *Materials Research Society Meeting,* San Francisco, USA, 2014.

### Poster Presentations

[1] T. Rossow, D. Steinhilber, S. Seiffert, R. Haag
"Controlled Synthesis of Degradable Cell-Laden Microgels by Radical-Free Gelation in Droplet based-Microfluidics" *Particles 2011,* Berlin, Germany, 2011.

[2] T. Rossow, S. Seiffert
"Controlled Synthesis of Cell-Laden Degradable Microgels by Radical-Free and Supramolecular Gelation in Droplet Microfluidics" *Smart Polymers*, Mainz, Germany, 2012.

# 8 Curriculum Vitae

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