Synthesis and Characterization of Temperature- and Light-Responsive Polymers and Block Copolymers

Dissertation zur Erlangung des Grades
"Doktor der Naturwissenschaften"

im Promotionsfach Chemie
am Fachbereich Chemie, Pharmazie und Geowissenschaften
der Johannes Gutenberg-Universität Mainz

Florian D. Jochum

geboren in Mainz

Mainz, 2010

Die vorliegende Arbeit wurde unter der Betreuung von PD Dr. habil. XXX in der Zeit vom März 2007 bis Juni 2010 am Institut für Organische Chemie der Johannes Gutenberg-Universität Mainz angefertigt.

Dekan: Professor Dr. XXX

Erster Berichterstatter: PD Dr. habil. XXX

Zweiter Berichterstatter: Professor Dr. XXX

Tag der mündlichen Prüfung: 21.07.2010

Contents

Abbreviations

Å	**Ångström**
AIBN	**azobisisobutyronitrile**
ATR	**attenuated total reflection**
ATRP	**atom transfer radical polymerization**
CMC	**critical micelle concentration**
CMT	**critical micelle temperature**
CTA	**chain transfer agent**
DLS	**dynamic light scattering**
DMF	**dimethylformamide**
DMSO	**dimethylsulfoxide**
DNA	**deoxyribonucleic acid**
FD-MS	**field desorption mass spectrometry**
FT	**fourier transform**
GPC	**gel permeation chromatography**
IR	**infrared radiation**
LCST	**lower critical solution temperature**
M_n	**molecular weight (number average)**
M_w	**molecular weight (weight average)**
NMP	**nitroxide mediated polymerization**
NMR	**nuclear magnetic resonance**
POEGMA	**poly(oligo(ethylene glycol) methyl ether methacrylate)**
PEG	**poly(ethylene glycol)**
PEO	**poly(ethylene oxide)**
PFP	**pentafluorophenol**
PNIPAM	**poly(*N*-isopropyl acrylamide)**
PPFPA	**poly(pentafluorophenyl acrylate)**
RAFT	**reversible addition-fragmentation chain transfer**
SPR	**surface plasmon resonance**
TEM	**transmission electron microscopy**
UV	**ultra-violet (light)**
Vis	**visible (light)**

Introduction

Polymers

A polymer is defined as a large molecule composed of repeating units typically connected by covalent chemical bonds. While the word “polymer” in popular usage suggests plastic, the term actually refers to a huge class of natural and synthetic materials. Due to the extraordinary range of properties accessible in polymeric materials, they have come to play an essential role in everyday life - from plastics and elastomers to natural biopolymers such as DNA or proteins.

Similar to classical organic compounds, the number of different polymers today is almost endless. In recent years, more and more polymers with various structures, functional groups and variable molecular weights have been synthesized for different versatile optical, thermal, technical or electrical applications. Especially, materials based on functional polymeric amphiphiles are of great interest as they allow a self-organization in selective solvents. This enables the access to medical applications such as drug delivery.

Recently, polymeric micelles found great interest in the area of self-organized materials and as such, the term “micelle” will be discussed in the following.

Micelles

A micelle, derived from the latin word “mica” (little lumps), is a small aggregate formed from amphiphilic molecules or surfactants dispersed in a liquid.[1,2]

The formation of micelles occurs spontaneously due to the tendency of the amphiphile to undergo phase separation. A typical amphiphile forms an aggregate from aqueous solution with the hydrophilic “head” regions in contact with the surrounding solvent, forcing the hydrophobic single tail regions in the centre of the micelle. This type of micelle is called a normal phase micelle. Inverse micelles have the head groups at the centre and the tails extending out (see figure 1).[1,2]

Micelles are in general of spherical shapes. Other phases, including ellipsoids, cylinders, and bilayers are also known. The shape and size of a micelle is a function of the molecular geometry and the solution conditions such as concentration, temperature, pH and ionic strength.[2,3]

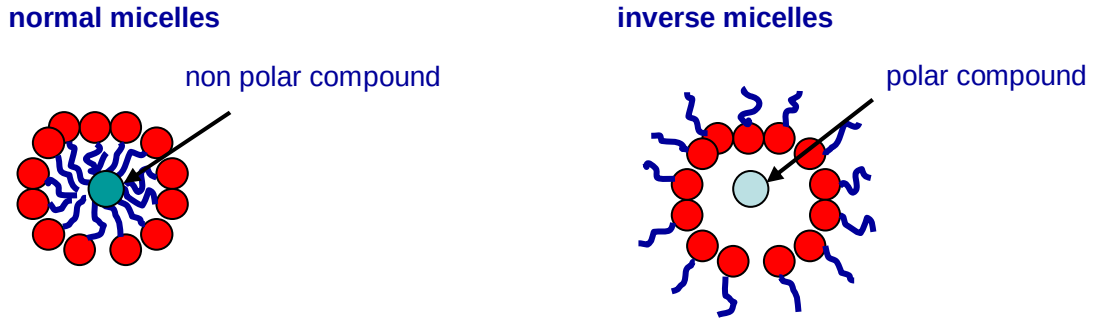


Figure 1. Schematic illustration of normal and inverse micelles.

Micelles can only form when the concentration of the surfactant is higher than the critical micelle concentration (CMC), and the temperature of the system is higher than the critical micelle temperature (CMT).[2,3] The formation of micelles is a thermodynamic phenomenon: micelles form spontaneously because of a balance between entropy and enthalpy. In water, the hydrophobic effect is the driving force for the formation of micelles, despite the fact that assembling surfactant molecules together reduces their entropy.

At very low surfactant concentrations, only solubilized molecules are present in the solution. As the concentration of the surfactants is increased, a concentration is reached at which the unfavorable entropy consideration, derived from the hydrophobic ends of the molecules, becomes dominant. At this point, the hydrophobic chains of the amphiphiles are forced away from the water. Therefore, the amphiphiles start to form micellar structures. Broadly speaking, above the CMC, the entropic penalty of assembling the surfactant molecules is less than the entropic penalty of caging the surfactant monomers with water molecules (see figure 2).[1,2]

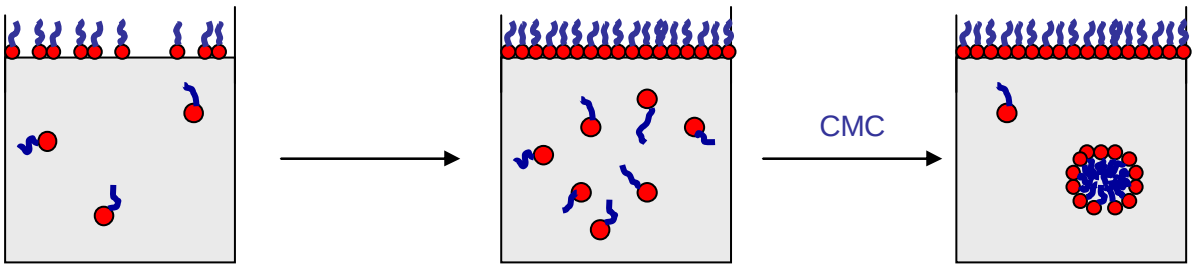


Figure 2. Formation of micelles above CMC.

As already mentioned, the CMC is an important parameter for the characterization of micelles formed from amphiphilic molecules. Many physical parameters are changed upon reaching the CMC such as the osmotic pressure π, the light scattering intensity I_{SC} or the surface tension (see figure 3).[1,2]

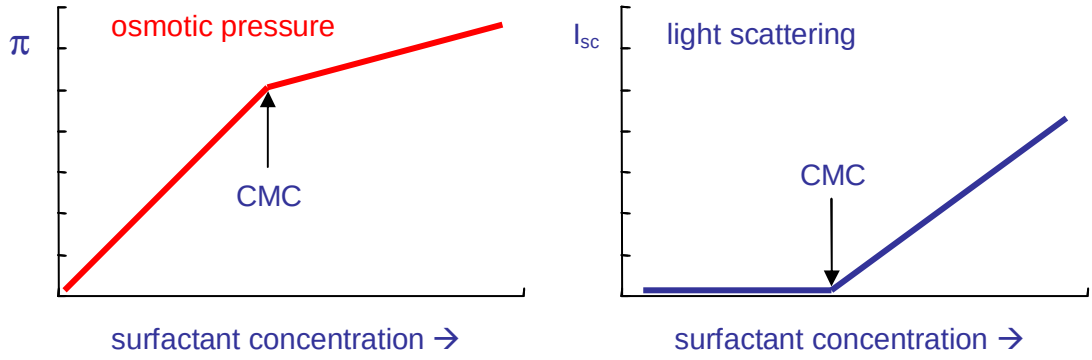


Figure 3. Change of the of the osmotic pressure π and of the light scattering intensity I_{sc} at the CMC.

All these methods are applied for the determination of the CMC by carrying out measurements on a series of different surfactant concentrations. The CMC is obtained from the intersection of the straight lines from the measurements.

Recently, polymeric amphiphiles capable to form polymeric micelles in solution are the focus of current research.

Polymeric micelles

The structural variety of low molecular weight amphiphiles such as lipids and surfactants is very limited.

In contrast, manifold polymeric structures based on diblock and triblock copolymers, as well as branched, hyperbranched polymers or polymers with "brush-like" structures have been reported (see figure 4).[2,3] The hydrophilic-hydrophobic (lipophilic) balance in polymeric amphiphiles can greater be modified towards low molecular weight compounds.

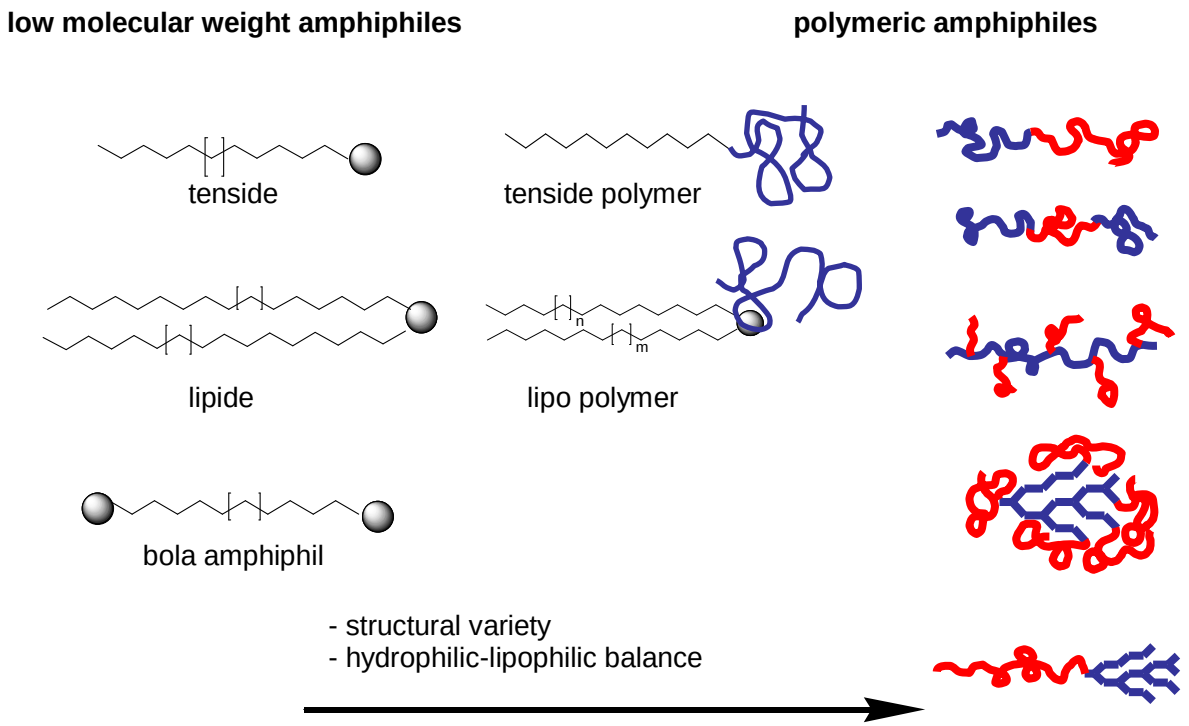


Figure 4. Increase of the structural variety and of the hydrophilic-lipophilic balance from low molecular weight amphiphiles to polymeric amphiphiles.

In analogy to their behavior in bulk, diblock copolymers self-assemble in block-selective solvents (e. g. in water), which solubilize only one but not the other block, forming micelles of various shapes.[2-5] If the soluble block is predominant, the insoluble block aggregates to form a spherical micelles. Micelles have typically a core-shell structure. The hydrophobic core of the polymeric micelles in water, can encapsulate small molecules such as therapeutic drugs, while the hydrophilic shell provides interactions with the solvent and guarantees the nanoparticles stability (see figure 5). This makes these structures interesting for biomedical applications, such as drug delivery. The size of polymeric micelles usually ranges from 10 to 100 nm.[3]

Micelles can be characterized by several individual techniques, such as dynamic light scattering (DLS), transmission electron microscopy (TEM), fluorescence spectroscopy, differential scanning calorimetry (DSC), atomic force microscopy (AFM) or nuclear magnetic resonance spectroscopy (NMR).[2]

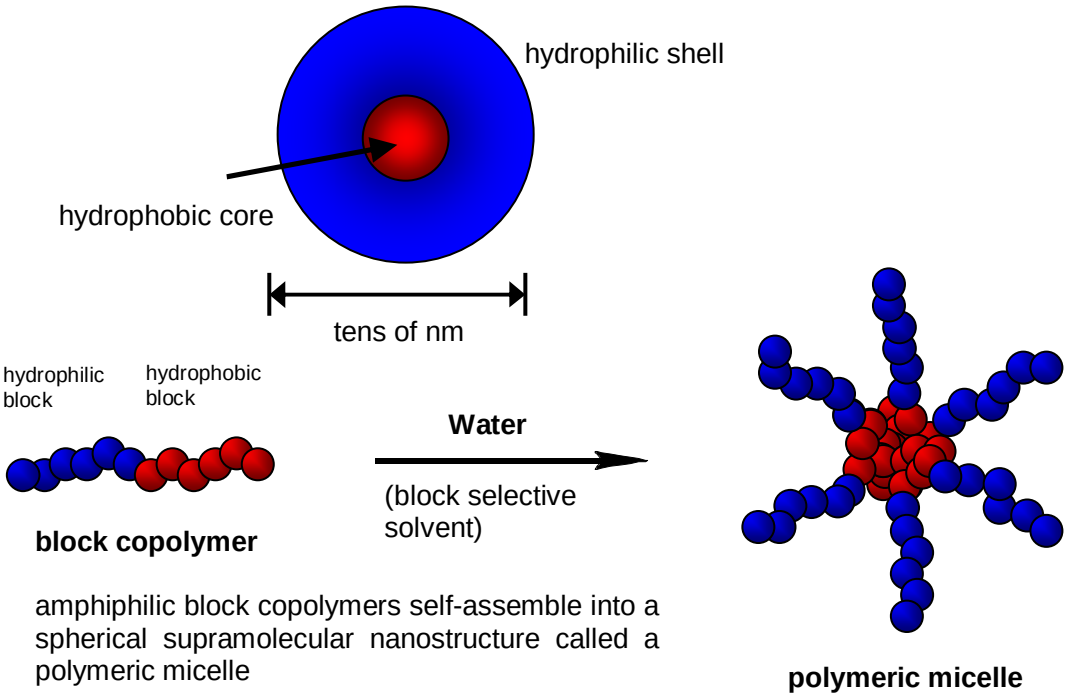


Figure 5. Self-assembly of amphiphilic block copolymers into polymeric micelles.

As already mentioned, amphiphilic block copolymers are prone to form polymeric micelles. A block copolymer is in generally defined as follows.

Block copolymers

Block copolymers are a certain class of polymers, also known as "soft materials". Independent from the synthetic procedure, they can simply be considered as being formed by two or more chemically homogenous polymer segments joined together by covalent bonds.[2,3] In the simplest case of two distinct monomers, conventionally named A and B, linear diblock (AB), triblock (ABA), multiblock or star-block copolymers can be prepared. The microphase morphology of diblock copolymers in bulk has been the subject of several theoretical and experimental investigations in recent decades. This self-assembly process is forced by an unfavorable mixing enthalpy and a small mixing entropy, while the covalent linkage connecting the blocks prevents macroscopic phase separation.

Stimuli-responsive polymers and block copolymers have found great interest in academia and applied science, recently. In the next few lines, stimuli-responsive polymers will be introduced and some examples will be given.

Stimuli-responsive polymers

Stimuli-responsive polymers are polymers that undergo physical or chemical changes in response to small external variations in the environmental conditions.[6,7] Such polymers can be widely found in living systems. Proteins, polysaccharides and nucleic acids can be regarded as typical stimuli-responsive polymers. The basic principle of a stimuli-responsive polymer consists in the recognition of an external signal, the evaluation of the magnitude of this signal and then in changing its chain conformation in response to the signal. The stimuli can be divided in either physical or chemical stimuli.

Typical chemical stimuli are pH changes, ionic strength or the addition of chemical agents.[6] At the molecular level, these stimuli will modulate the interactions between polymer chains or between polymer chains and the solvents. Physical stimuli can be temperature changes, electric or magnetic field variations or mechanical stress.[6] In general, such stimuli will change the molecular polymer-solvent interactions at critical onset points. Some polymers can combine two or more stimuli-responsive properties, while two or more signals can be simultaneously applied. These systems are named dual- or multi-responsive polymers, respectively.

Biochemical stimuli have been considered as a certain category, which involves the responses to antigens, enzymes, ligands or other biochemical agents.[6,7]

Thermoresponsive polymers

Similar to low molecular weight compounds, the solubility of most synthetic macromolecules increases when heated. However, there exist some water-soluble polymers that separate from solution upon heating. This unusual property is referred to an inverse temperature-dependent solubility.

The characteristic of polymers which dissolve when cooled and phase separate when heated is known as a lower critical solution temperature (LCST).[6,8] This temperature corresponds to the region in the phase diagram where the enthalpic contribution of the water hydrogen bonded to the polymer chain becomes less than the entropic gain of the whole system (see figure 6). The LCST is largely dependent on the hydrogen bonding capacities of the repeating units of a water-soluble polymer.[6]

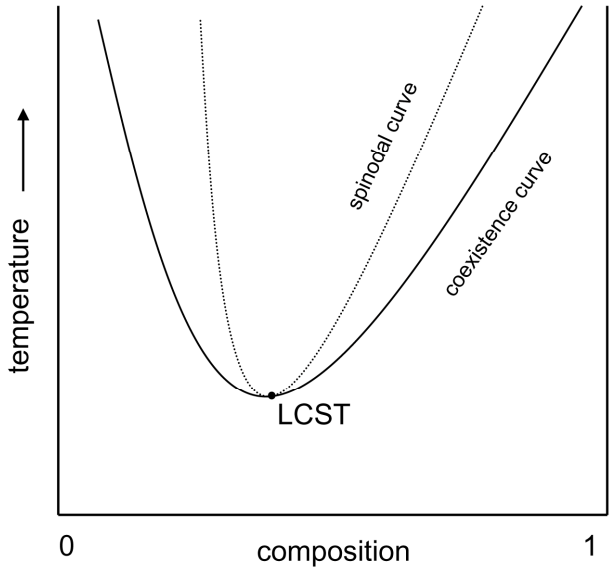


Figure 6. Plot of a typical polymer binary solution phase behavior including a LCST.

The simplest explanation is derivated from thermodynamics: the dissolution enthalpy ΔH due to the hydrogen bonding of the basic sites on the polymer with the solvent favors dissolution. However, the entropic organization ΔS of the solvent required to achieve this hydrogen bonding is unfavorable. The free energy of dissolution is equal to ΔH-TΔS. Following this principle, it can change from negative (favorable) to positive (unfavorable) by increasing the temperature of the system. Thus, the LCST behavior of polymers is favored in strongly interacting solvents such as water.[6]

In contrast to their biological counterparts, synthetic polymers often redissolve when cooled. This makes these materials more versatile than proteins, which do not "renature". Further, the temperature, at which the phase separation of a synthetic polymer from solution occurs, can be changed by varying the polymeric structure. Thermoresponsive polymers undergo reversible conformational or phase changes in response to negligible variations of the temperature (see figure 7).[6] Similar to the behavior in nature, the bulk response of a thermoresponsive polymer is usually due to multiple interactions such as loss of H-binding.

The quality of the response can be monitored by the cloud point temperature and by calorimetric measurements.[6] The coupling of calorimetry with cloud point measurements have been demonstrated to be a successful approach for the explanation of the phase transition temperature.

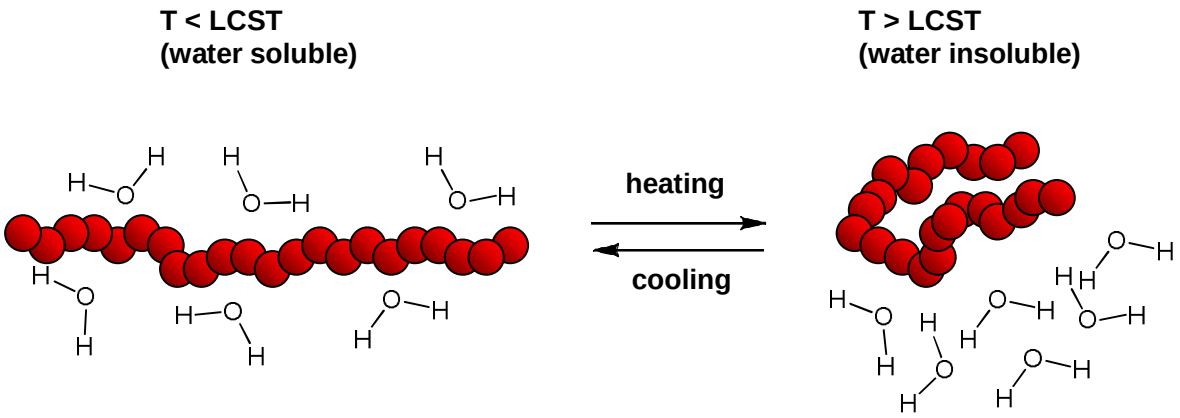


Figure 7. Reversible conformational changes in response to variations of the temperature of a thermoresponsive polymer.

The investigation of the phase transition by a controlled increase of the temperature followed by redissolution due to a controlled cooling process is very important to understand the LCST phenomenon. In general, most of the turbidity curves show a hysteresis. The phase transition can either be reversible, with hysteresis or with a gradual rather than an abrupt change. Poly(*N*-isopropylacrylamide) (PNIPAM) exhibits a very sharp transition when heated, but a broad hysteresis can be observed in the cooling period (see figure 8).[6,8,9]

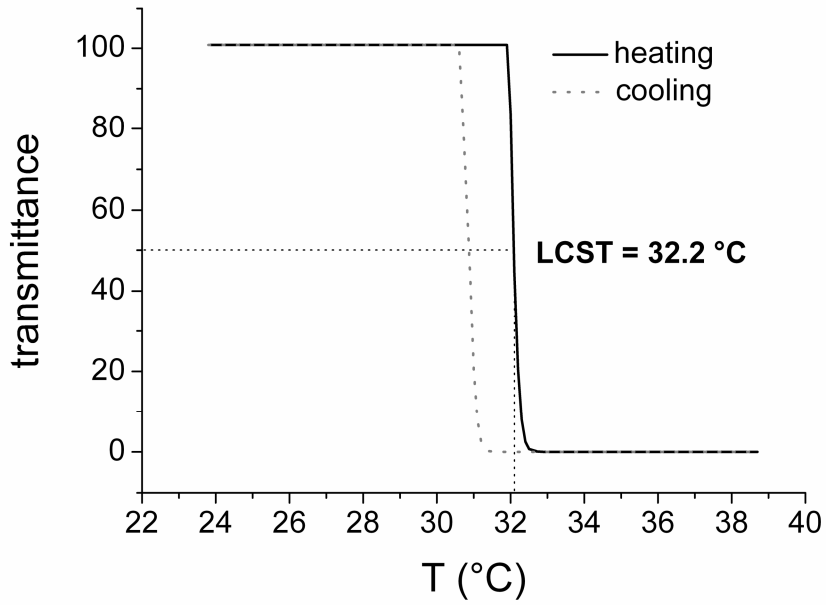
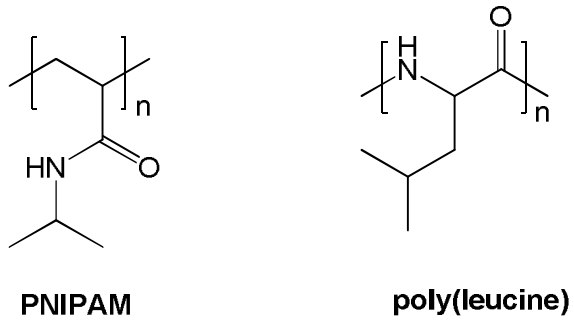


Figure 8. LCST curve of PNIPAM showing hysteresis between heating and cooling.

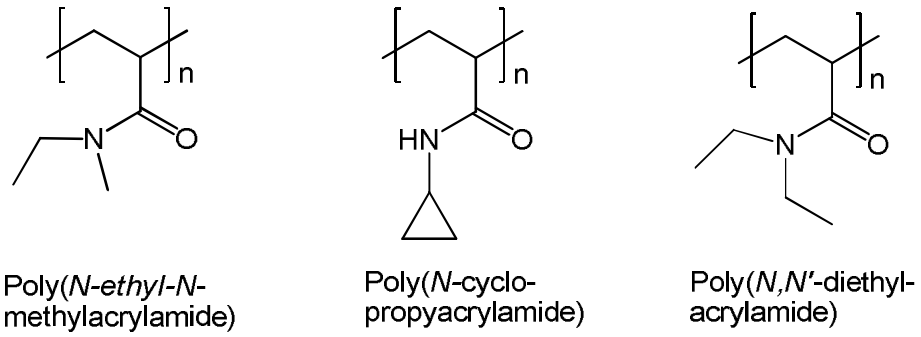
Below the LCST, the coiled structure in PNIPAM is favored. This structure allows a maximum interaction between the polymer and the water. In systems where strong hydrogen

bonding is possible, such interactions lower the free energy of dissolution considerably. At higher temperatures, the hydrogen-bonding effect weakens. As a consequence the entropy-controlled "hydrophobic effect", the tendency of the system to minimize the contact between water and hydrophobic surfaces increases. This process is followed by the transition from a coiled to a denser globule structure.[6] Indeed the phase transition temperature is dependent from several parameters such as the molecular weight, the concentration, salt concentration, tacticity, incorporation of co-monomers or the nature of the end group. The most studied synthetic thermoresponsive polymer is PNIPAM, which undergoes a sharp transition in water at 32°C, changing from a hydrophilic state below this temperature to a hydrophobic state above it. PNIPAM can be regarded as a simple model for a protein.[6,8,9] From the chemical point of view, PNIPAM is homologous with poly(leucine) (see scheme 1). However, in contrast to poly(leucine), PNIPAM has a nonpolar backbone and contains amide groups in its side chain. Poly(leucine) includes peptide groups in its backbone and has entirely nonpolar side chains.



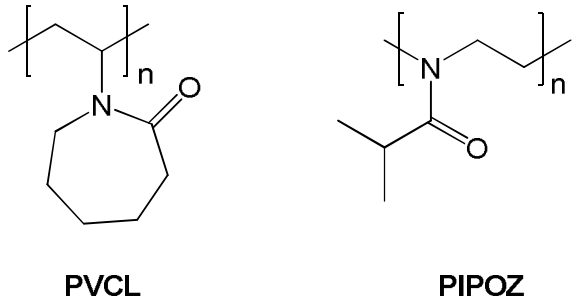
Scheme 1. Chemical structures of PNIPAM and poly(leucine)

As mentioned above, the origin of the "smart" behavior in PNIPAM, arises from the entropic gain as water molecules associated with the side chain isopropyl moieties are released into the bulk aqueous phase as the temperature increases above the LCST. The thermodynamic properties of aqueous PNIPAM solutions have been thoroughly investigated.[6] The transition from coil to a collapsed structure by increasing the temperature resembles the transition of a protein from unfolded to folded structures. Research in the field of thermosensitive polymers has been intensively focused on PNIPAM, but some other *N*-substituted poly(acrylamides) and poly(methacrylamides) exhibit a similar behavior in aqueous solution.[6] Poly(*N*-ethyl-*N*-methylacrylamide),[10] poly(*N*-cyclopropylacrylamide) (PNCPAM)[11] and poly(*N*,*N*'-diethylacrylamide)[12] homopolymers have been reported to display a LCST in the range between 30-56°C (see scheme 2).



Scheme 2. Chemical structures of thermoresponsive poly(acrylamides).

Polymers bearing amide groups are known to be the largest class of thermoresponsive polymers. Besides, poly(*N*-vinylcaprolactam) (PVCL) is a very important water-soluble polymer, which undergoes under warming a phase separation from aqueous solution (see scheme 3).[6,13] It has a repeating unit that consists a cyclic amide with the amide nitrogen directly attached to the hydrophobic polymer backbone. In contrast to thermosensitive poly(*N*-alkylacrylamides), it does not produce small amide derivatives upon hydrolysis. This feature, combined with its overall low toxicity, high complexing ability and good film forming properties enables its use in many industrial and biomedical applications.[6] Aqueous solutions of PVCL show a phase transition behavior at 31°C.[13] The LCST of PVCL shifts to lower values for higher molecular weights of the polymer.



Scheme 3. Schematic structures of poly(N-vinylcaprolactam (PVCL) and poly(2-isopropyl-2-oxazoline) (PIPOZ).

The repeating unit of poly(2-isopropyl-2-oxazoline) (PIPOZ) is structural isomeric to that of PNIPAM (see scheme 3).[6] Indeed, aqueous solutions of PIPOZ show a cloud point temperature. The cloud point of PIPOZ with a molecular weight of M_n = 16700 g mol^{-1} ranges from 36 to 39 °C depending on polymer concentration, as revealed by several turbidity studies.[14]

Another group of thermosensitive polymers includes the polyethers. Poly(propylene oxide) (PPO) is a most intensively investigated thermosensitive polymer.[6,15] However, only PPO oligomers are soluble in cold water.

Polymers and copolymers based on oligo(ethylene glycol) side chains were promised to become popular materials for biotechnological applications.[6,16] Copolymers poly(2-(2'-methoxyethoxy)ethyl methacrylate-*co*-oligo(ethylene glycol) methacrylate) with an average 5 percent of oligoether per chain exhibit a thermoresponsive behavior comparable to PNIPAM.[17] These class of novel thermoresponsive copolymers are very versatile for many applications in material science and technology. They might bear a great potential in drug delivery systems as some of them are known to degrade to harmless by-products during hydrolysis.[6]

Lightresponsive polymers

Besides the aforementioned temperature as a stimulus, light has attracted great attention, recently. Light can be localized in time and space and has therefore emerged as a trigger to release active molecules (drugs) from block copolymer micelles.[18-20] The general principle for a light-induced release is as follows. Firstly, small molecules are incorporated in the core of lightresponsive block copolymer micelles. Upon irradiation, a change in the nature of the core-forming block occurs which further leads to a disruption of the micelles. As a consequence, the trapped compound (drug) is released (see figure 9). In the case of amphiphilic block copolymer micelles, light induces a change in the polarity of the core-forming block, which renders the block copolymer totally soluble. This kind of disruption can either be irreversible or reversible depending on the kind of used photosensitive moiety.

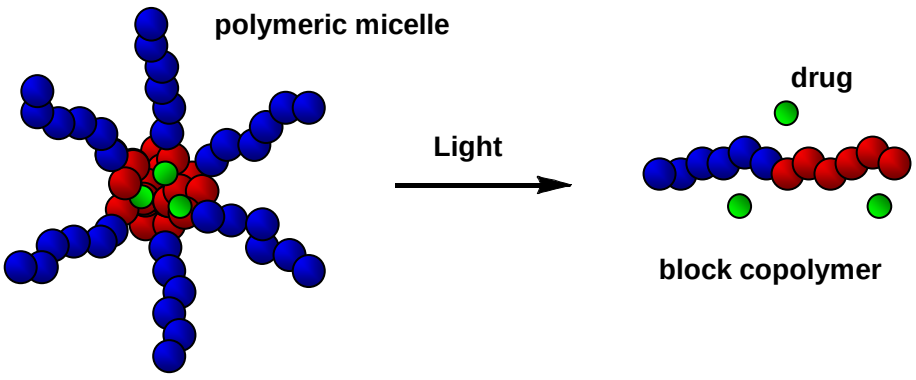


Figure 9. Schematic principle of light controlled (irreversible) disruption of a polymeric micelle accompanied with drug release.

Some examples for amphiphilic lightresponsive polymers have been described by Zhao and co-workers, recently.[21-23] Following the same strategy, polymers containing one hydrophilic poly(ethylene oxide) segment linked to a hydrophobic polymethacrylate block bearing photolabile groups were synthesized. Pyrenylmethyl, *o*-nitrobenzyl or coumarin esters were used as photocleavable groups. The irradiation with UV-light induced an irreversible cleavage of the side chromophores, unmasking the protected carboxylic acid functions. As a consequence, the irradiated block copolymer was completely hydrophilic and became soluble in water. Transferred on polymeric photocleavable micelles in water, the light stimulation would result in the irreversible disruption of the aggregates and a subsequent release of the previously encapsulated molecules (drugs) would follow. A special example of an irreversible light induced disruption has been reported by Stupp and coworkers.[24,25] They investigated photosensitive peptide oligomers containing *o*-nitrobenzyl ester groups.

Azobenzene moieties have been recognized early as suitable candidates for reversible light-induced micellation of block copolymers.[26] Azobenzene chromophores undergo easily a reversible *trans*-*cis* isomerization of their nitrogen-nitrogen double bond. The *trans* isomer of azobenzene can be converted into the *cis* upon UV-light irradiation and the back isomerization can be activated by visible light exposure. Zhao and co-workers developed a micellar system which can be disrupted upon UV-light irradiation and reforms itself under visible light.[26,27] Therefore, a block copolymer containing one poly(*t*-butyl acrylate-*co*-acrylic acid) sequence connected to a poly(methylacrylate) bearing azobenzene side group chromophores was synthesized. The formation of core-shell micelles and/or vesicles was observed for the block copolymer in a dioxane/water mixture. Both morphologies could be disrupted upon UV-light irradiation. The aggregates reform themselfs after exposure to visible light. This reversible disruption-reformation process was explained by the change in the hydrophilic-hydrophobic balance of the block copolymer induced by the photoisomerization of the azobenzene side groups. Matyjaszewski and co-workers recently proposed the use of photosensitive spiropyran moieties instead of azobenzene, in order to enhance the efficiency of a reversible photo-induced micellar dissociation-association process.[28] For that, poly(ethylene oxide)-*block*-poly(methacrylate) whose methacrylate block was bearing spiropyran side chains was synthesized. The hydrophobic spiropyran moieties undergo photoisomerization upon irradiation with UV-light into their hydrophilic merocyanine counterparts. In analogy to azobenzene, the reverse process is triggered by visible light. The isomerization of spiropyran moieties is accompanied by a deep change of the hydrophilic to hydrophobic balance in the block copolymer. A consequence is a reversible disruption of

micelles. Following again the work on azobenzene-containing block copolymers, some interesting studies on the behavior of micellar nano- and microaggregates have been reported. [29-34]

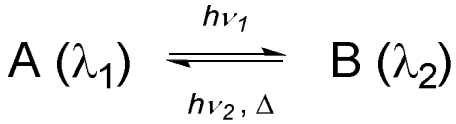
Thermo- and lightresponsive polymers

Multi-responsive systems have been attracting great interest recently as they offer the possibility to tune their properties in many different ways. Lightresponsive polymers are often combined with temperature as an additional stimulus. A first example of thermo- and lightresponsive copolymers was reported in 1988 by Irie and coworkers.[35] They synthesized PNIPAM copolymers containing azobenzene moieties. The LCST of the PNIPAM copolymer containing 2.7 mol% azobenzene increased upon UV-light irradiation from 21°C for the *trans* form to 27°C for the *cis* form. So far, other examples for thermoresponsive polymers and block copolymers based on azobenzene chromophores have been published.[36-41] A particular example, showing a greater change in the LCST upon illumination involves *o*-nitrobenzyl polyacrylates and a thermoresponsive poly(ethoxy triethyleneglycol)acrylate in a block copolymer structure.[42] In this case, the LCST could be increased by more than 10°C. However, these changes in the phase transition temperature were irreversible due to the cleavage of *o*-nitrobenzyl esters into carboxylic acids.

As already discussed in the previous chapter, the reversible photoreaction of lightresponsive molecules is important for many responsive polymeric materials. In the following, the term "photochromism" will be introduced and some examples for photochromic compounds will be given.

Photochromism

A photochromic organic compound is a reversible dye under photochemical control.[43] Photochromism can be defined as a reversible transformation of chemical species, induced in one or both directions by electromagnetic radiation, between two states (A and B) having light absorptions in different regions (see scheme 4).



Scheme 4. Photochromism between two states A and B.

In general, the photochromic reaction involves a reversible transformation between two species with B having at least one absorption band appearing at longer wavelength λ than those of A. UV-light in the range between 300 to 400 nm is in general used for initialization of the photochromic reaction. Most photochromic systems are established to be unimolecular reactions (A → B). However, the reversibility is the major criterion for photochromism. The back reaction (B → A) can occur predominantly by a thermal mechanism, as is the case for spiropyrans, spirooxazines and chromenes. For these systems, the thermally driven back reaction can be accompanied by one that is photochemically driven. However, in most cases the thermal reaction predominates. For other systems, the photochemically induced forms (B) are thermally stable. For such systems, e. g. fulgides or arylethenes, the back reactions (B → A) occur predominantly photochemical. The photochromic transformation and the observed spectral changes or changes in physical or chemical behavior are related to the modifications of the geometry of the system and its electronic distribution.

Ordinarily, photochromism and its various characteristics can be defined according to the type of application at which they are targeted. Two general types of applications can be defined:

1. Applications, that are directly dependent on the color change caused by the molecular and electronic structures of the two species (A, B) and their corresponding absorption or emission spectra.

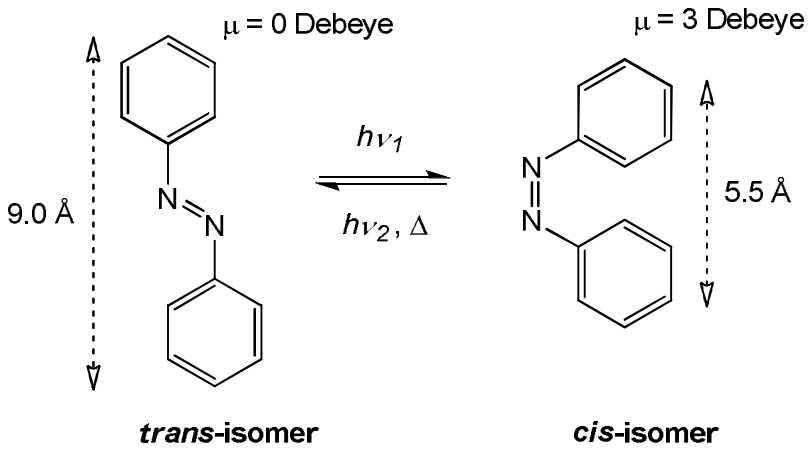
2. Applications that are dependent on changes in the physical or chemical properties that occur during the photochromic reaction. Examples of such properties are conductivity, refractive index, electrical moment, dipole moment, dielectric constant, chelate formation, ion dissociation, phase transitions, solubility, and viscosity. Certain physical changes that occur when the photochromic moiety is chemically attached to the macromolecular backbone of polymers are of special interest in this dissertation.

In the following, the most important photochromic molecules will be presented.

Azobenzene

Azobenzene is a chemical compound composed of two phenyl rings linked by a N=N double bond.[44-48] The term “azobenzene” or simply “azo” is often used to refer to a wide class of molecules that have an azobenzene structure, with different chemical functional groups extending from the phenyl rings. These compounds should be formally referred to “diazenes”. The diazenes strongly absorb light and were historically used as dyes in industry (“aniline yellow”). Azobenzene was first described by Nobel in the year 1856 as "gelblich-rote kristallinische Blättchen".[49] Its original preparation is similar to the modern one. According to the old method, nitrobenzene is reduced by iron filings in the presence of acetic acid. In modern synthesis, zinc is uses as a reductant in the presence of a base.[50]

The most fascinating property of azobenzene and its derivatives is the photoisomerization of *trans* to *cis* (see scheme 5). The two isomers can be switched with particular wavelengths: UV-light, which corresponds to the energy gap of the π-π* (S2 state) transition, for *trans*-to-*cis* conversion, and visible light, which is equivalent to that of the n-π* (S1 state) transition, for *cis*-to-*trans* isomerization.[45] For several reasons, the *cis*-isomer is less stable than the *trans*-isomer. Thus, *cis*-azobenzene will thermally relax back to *trans*-azobenzene via *cis*-to-*trans* isomerization. The *trans*-isomer is more stable by approximately 50 kJ/mol, and the barrier to photo-isomerization is approximately 200 kJ/mol. The photo-isomerization of azobenzene occurs extremely rapid within picoseconds. The rate of the thermal back-relaxation is strongly dependent on the compound and is usually in the range of several hours. The photo-isomerization of azobenzene is accompanied with significant changes in the geometry of the molecule and the dipole moment μ.[45]

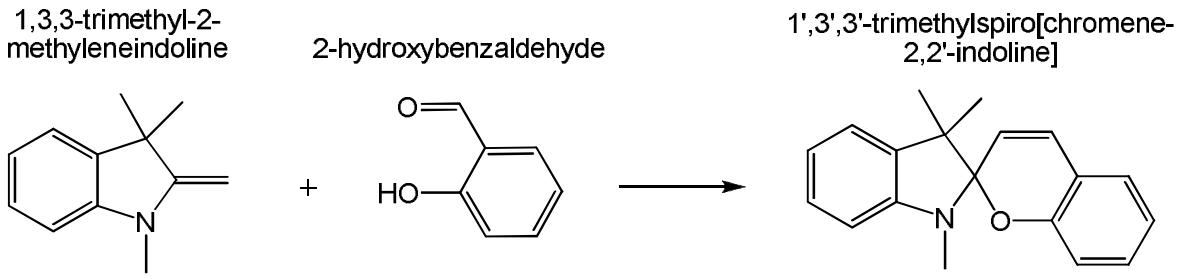


Scheme 5. Azobenzene photoisomerization accompanied with a change of the dipole moment μ.

The conversion from *trans*-to-*cis*-azobenzene decreases the distance between the 4 and 4' ring positions from 9.0 to 5.5 Å, however the dipole moment increases from 0 to 3 Debye.[45]

Spiropyran

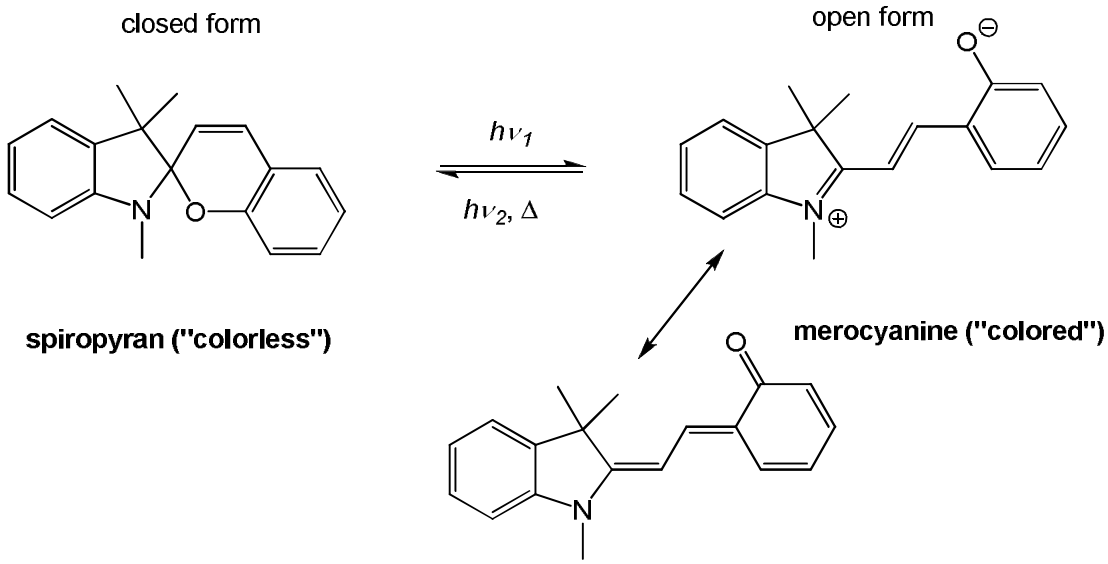
"Spiropyrans" refers in general to a substituted 2*H*-pyran having a second ring system, usually heterocyclic, attached to the 2-carbon atom of the pyran in a spiro manner.[43] The indolinospiropyrans is the one of the most studied of all photochromic compounds. The spiropyrans, their photochromism, and their applications have been extensively reviewed.[51,52] Spiropyrans can be synthesized in several different ways. One of the most common methods, is the condensation of a 2-alkyl heterocyclic quaternary salt or the corresponding methylene base with a 2-hydroxy unsaturated aldehyde grouping (see scheme 6).[43] These intermediates have given a broad assortment of spiropyran classes.



Scheme 6. Synthesis method of a spiropyran compound.

A reversible photocoloration is attributed to an equilibrium between the spiropyran ("closed", "colorless") form and a merocyanine ("open", "colored") form, as shown in scheme 7 for an indolinospirobenzopyran. The merocyanine itself represents an equilibrium mixture of geometrical conformations, and its electronic distribution varies from highly zwitterionic to an essentially nonionic ortho-quinoidal structure.

The existence of the spirobenzopyran-merocyanine equilibrium implies that in principle a dimethinemerocyanine could be closed to a pyran isomer. This would be an example for a "reverse" photochromism. However, many merocyanines can be irreversibly photobleached by reaction with some added reagent.



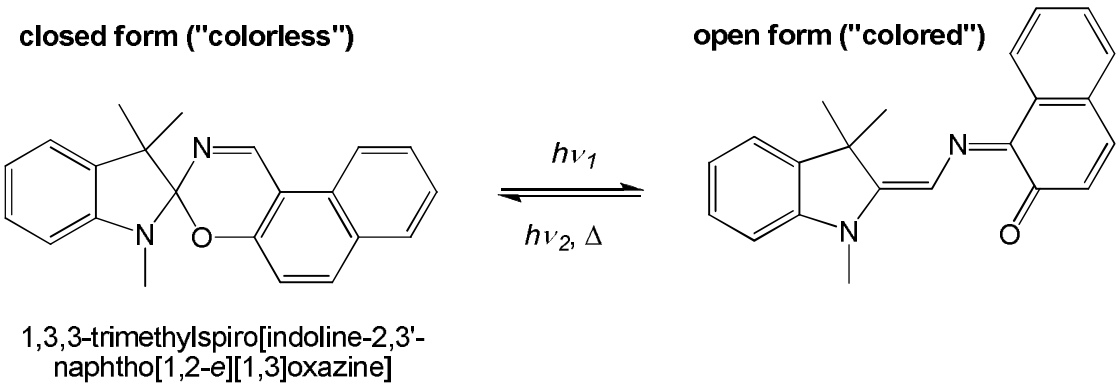
Scheme 7. Photoisomerization reactions of an indolinospiropyran compound.

Spiropyrans are promised to be used for optical recording, three-dimensional optical memories, and holography.[43] Today, spiropyrans are commercially used in moderate quantities such as for exposure indicators in photolithographic plates, for microimage recording, and in a most interesting application for fluid-flow visualization. For these uses, fatigue is not an important limitation. In addition, relatively small amounts of spiropyrans are used in printing inks for T-shirts and in toys.

Spirooxazine

Photochromic spirooxazine compounds are molecules containing a condensed ring-substituted 2*H*-[1,4]oxazine in which the number 2 carbon of the oxazine ring is involved in a spiro linkage.[43] The photochromism of spirooxazines implies the heterolytic or homolytic

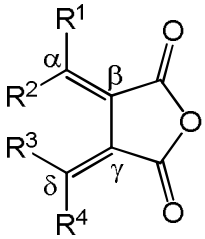
cleavage and reformation of the carbon-oxygen single bond of the oxazine ring. In solution, the parent indolinospironaphthoxazine (NISO) turns blue upon irradiation with UV-light and rapidly fades back to colorless when the activating radiation is removed. Only UV-A light (315-380 nm) is required for activation. The blue color is caused by the formation of the ring-opened merocyanine structure, which absorbs in the region of 600 nm. Spirooxazines show an excellent resistance towards light-induced degradation. This property, also called fatigue resistance, is considered to be due to photochemical stability of the oxazine molecule framework in the ring-closed form as well as in the open-ring form. The fatigue resistance of the spirooxazines has led to their successful use in various applications, e. g. in eyewear.[43] A typical structure of an indolinospironaphthoxazines derived from 1-nitroso-2-naphthol is illustrated in scheme 8.



Scheme 8. Photoisomerization reaction of an indolinospironaphthoxazines compound.

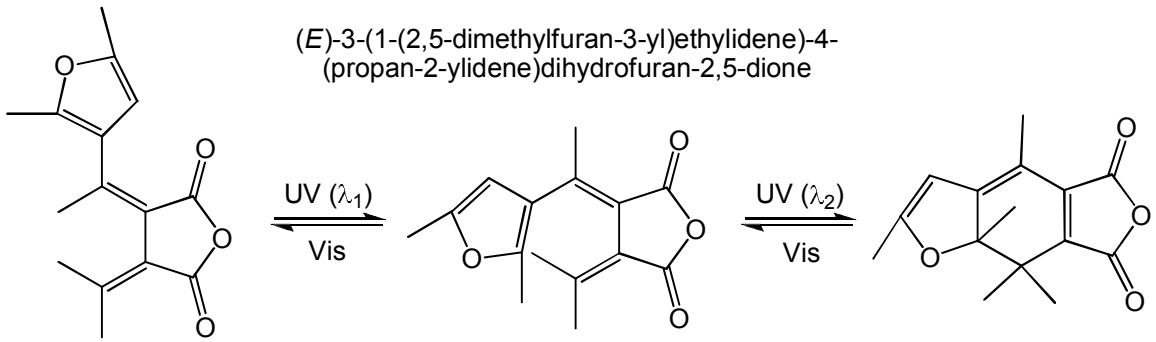
Fulgides and fulgimides

The fulgide family represents an important type of photochromic compounds.[43,52] The photochromism of some phenyl-substituted bismethylene succinic anhydrides in the solid state was fist discovered by Stobbe. [53]



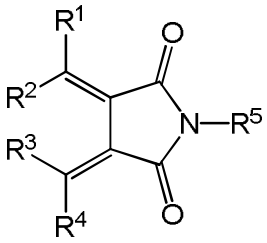
Scheme 9. Molecular structure of a fulgide.

In general, fulgides are synthesized by a condensation of aryl aldehyde or ketone with a substituted methylene succinate, followed by hydrolysis and a dehydration process. At least one of the four substituent groups on the fulgide is an aromatic ring, e. g. a phenyl group. The structure of the fulgide is constructed as a hexatriene unit that has two different isomers, a *Z* form and an *E* form based on the α,β-unsaturated double bond, R^3 and R^4 being the same. If one considers the two double bonds α,β and γ,δ substituted fulgides with four different groups, R^1, R^2, R^3 and R^4 in compound 1, four geometrical isomers, e.g. (*E*,*E*), (*E*,*Z*), (*Z*,*E*), and (*Z*,*Z*) structures, can exist (see scheme 9). When photochromic fulgides are irradiated with UV-light, they either isomerize from the *E* to *Z* conformation or photocycle depending on the wavelength of the incoming light (see scheme 10). The photocyclization reaction of phenyl fulgides occurs by a photochemical conrotatory process. However, the thermal electrocyclic ring-opening reaction is a disrotatory process in accordance with the Woodward-Hoffmann selection rules.[43]



Scheme 10. Photoisomerization reactions of a fulgide compound.

The first compound of the fulgide family developed by Heller was a succinimide which he called "fulgimide".[54] The fulgimides represent the most important fulgide derivatives (see scheme 11). They are reported to have photochromic properties similar to the parent fulgides, but the absorption bands show a hypsochromic shifts.[43] Fulgimides can be synthesized by the dehydration of succinamic acids, which are prepared by either the reaction of a fulgide with ammonia or primary amine, or by the reaction of a succinic half-ester with the Grignard salt of the amine. Some *N*-substituted fulgimides are prepared by reacting fulgimides with bromo or hydroxy compounds.

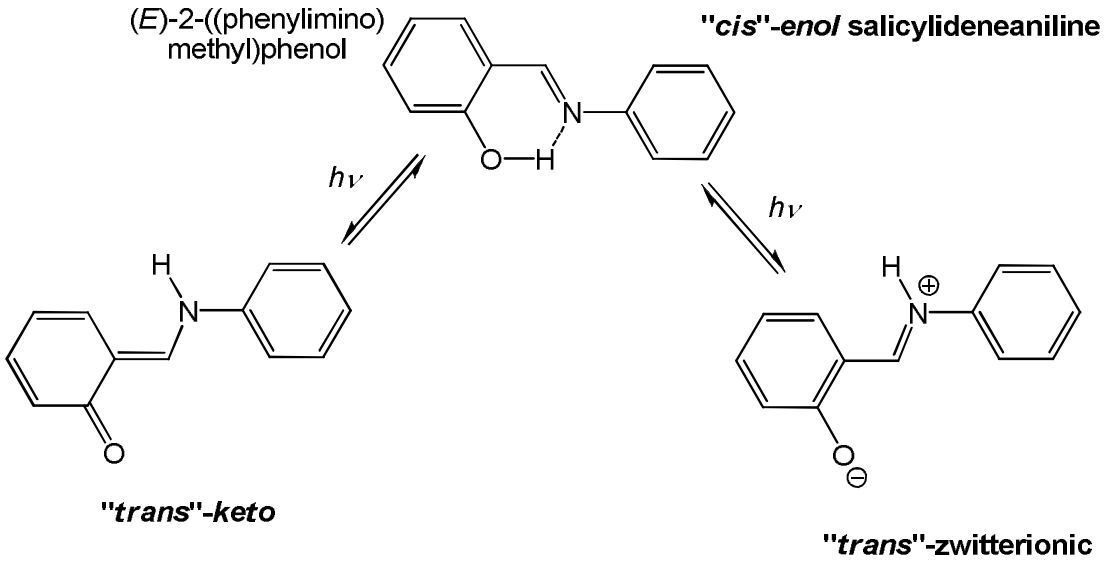


Scheme 11. Molecular structure of a fulgimide.

Based on the different classes of fulgide and fulgimide family compounds, many realistic and potential applications have been developed and suggested, e. g. actinometry, optical storage, optical data processing, nonlinear optical materials, optical waveguides, optical switches, security and printing applications and eyewear as well as leisure products.[43]

Salicylideneaniline

N-(2-hydroxy benzylidene) aniline, abbreviated as salicylideneaniline, has been known to exhibit photochromism both in its crystalline and solution phases (see scheme 12).[55-60] Salicylideneaniline and derivatives can be synthesized from an aromatic amine and salicylaldehyde by a nucleophilic addition reaction forming a hemiaminal, followed by dehydration.[58] It is generally agreed that photo excitation of salicylideneaniline is followed by a rapid proton transfer along the intramolecular hydrogen bond of the *ortho*-hydroxyl to the imine nitrogen to form the *cis* proton transferred product which isomerizes to the more stable *trans*-form around the C1-C7 and C=N bonds.[56]



Scheme 12. Photoisomerization reactions of a salicylideneaniline compound.

For the final photoproduct, two different structures have been proposed. The first one is originated from the full transfer of the proton (or more specifically, hydrogen) from the initial *enol* structure to give *cis*- and *trans-keto* forms, whereas, the other involves only a partial transfer to *cis* and *trans* zwitterionic structures.

The synthesis of polymeric materials combining photochromic and thermoresponsive properties is still a challenge. However, one has to find a suitable synthetic procedure to obtain defined molecular weights and architectures of these materials. In the following chapter, the RAFT polymerization technique as a method of choice will be introduced.

RAFT polymerization

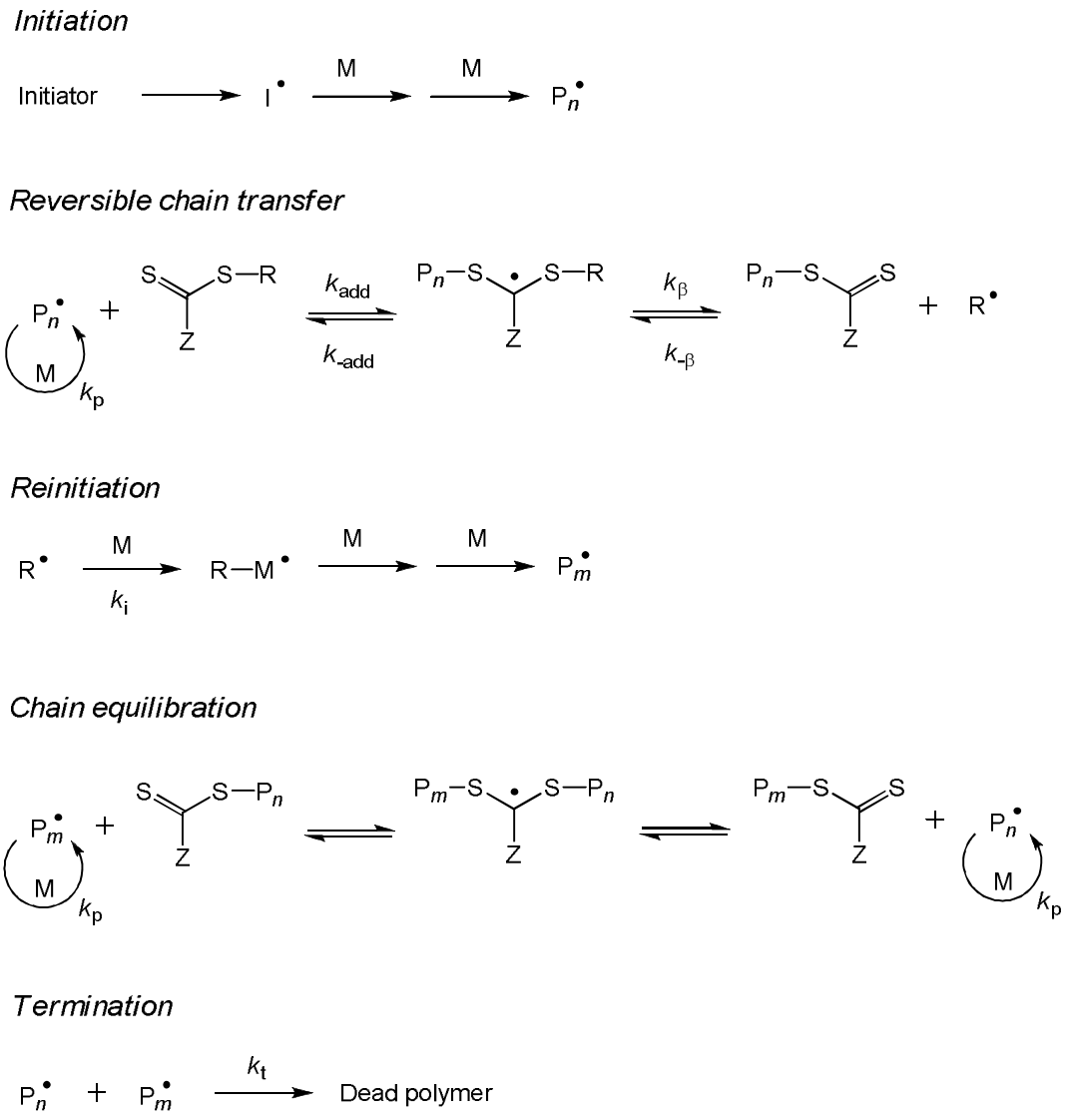
Radical polymerization is probably the most widely used processes for the commercial production of high-molecular weight polymers.[61] The main factors responsible for the preeminent role of radical polymerization are as follows (a) it can be used with a large variety of monomers, (b) it is tolerant towards a wide range of functional groups and reaction conditions, and (c) it is simple to perform and inexpensive in relation to competitive technologies. However, the conventional process has some notable limitations with respect to the degree of control over the molecular weight distribution, polymer composition and architecture.

The recent emergence of techniques for implementing living radical polymerization has provided a new set of tools that allow very precise control over the polymerization process. The versatility of the methods towards conventional radical polymerization is still maintained.[62-64] Recently, living radical polymerization techniques such as the nitroxide-mediated polymerization (NMP)[65], the atom transfer radical polymerization (ATRP)[66,67] and the reversible addition-fragmentation chain transfer (RAFT)[68] have received great attention. The NMP technique was devised in the early 1980s. The polymerization method has been exploited extensively for the synthesis of homopolymers and block copolymers with a narrow molecular weight distribution.[69-72] New developments have made NMP applicable to a wider, though still restricted range of monomers.[72]

ATRP is substantially a more versatile polymerization technique.[67,73] However, it requires unconventional initiating systems, such as copper salts with poor compatibility to the polymerization media. Additionally, the use of transition metals in ATRP might bear some problems with regard to biological uses of the resulting polymers. Substantial advances have been made to address this and other issues.

RAFT polymerization is one of the more recent entrants in this field and probably the most convenient and versatile. RAFT agents, also called chain transfer agents (CTA), were first reported in the literature in 1986.[74] However, their use in polymer synthesis to produce polymers with narrow molecular weight distribution was not published until 1995.[75] The use of xanthates to give RAFT agents was reported firstly in 1988[76,77] while the use of thiocarbonylthio RAFT agents in this context of conferring living characteristics on radical polymerization was communicated in 1998.[68] Thiocarbonylthio RAFT agents may act as chain transfer agents by a two-step addition fragmentation mechanism as illustrated in scheme 13.

In general, the RAFT mechanism[64] starts with a fragment of a radical initiator, such as a cyano-propyl-radical originating from AIBN, attacking the dithioester. The Z group has the purpose to stabilize the resulting carbon-based radical and encourage its formation through addition of the initiator fragment. The R group is chosen to stabilize an R-based radical and is often designed to resemble the monomer indented for polymerization. The following fragmentation is thus directed into expelling the R group, which starts the polymerization. The terminal radical of the propagating chain may then add onto another dithioester molecule, setting free its R group (or an already formed polymer chain) in turn, thus transferring the radical. In summary, the polymer chain is inserted between the R group and the dithioester (DTE), resulting in the structure R-polymer-DTE-Z. The RAFT process thus has a great potential in producing polymers with defined functional end groups.



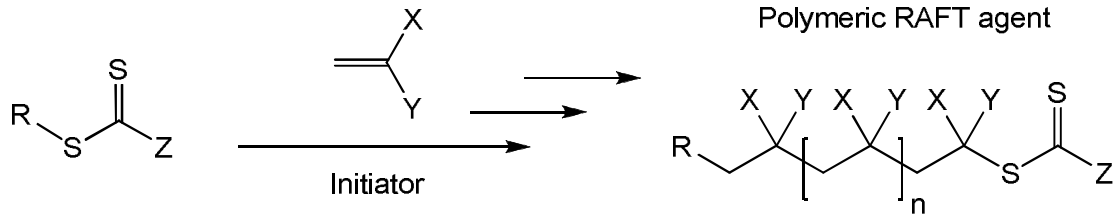
Scheme 13. Mechanism of RAFT polymerization.[64]

RAFT polymerization with thiocarbonylthio compounds has been found compatible with the majority of monomers polymerizable by free radical processes.[64] This includes (meth)acrylates, (meth)acrylamides, acrylonitrile, styrene and derivatives, butadiene, vinyl acetate, and *N*-vinylpyrrolidone. The RAFT polymerization is tolerant towards the functionality in monomer, solvent and initiator.

The essential features of the ideal RAFT polymerization (see scheme 14) can be summarized as follows[64,78]:

- RAFT polymerization can be performed by simply adding a chosen amount of an appropriate RAFT agent to a conventional free radical polymerization. Usually, the same monomers, initiators, solvents and temperatures can be used.
- RAFT polymerization possesses the similar characteristics like other living polymerization methods (e.g. anionic polymerization). Essentially, all chains begin their growth simultaneously and continue to grow until the monomer is consumed completely. Molecular weights increase linearly with conversion. Active chain ends are retained, thus allowing an access to end group functionalized polymers.
- Molecular weights in RAFT polymerization can be estimated using equation 1 where $[M]_0 - [M]_t$ is the monomer consumed and m_M is the monomer molecular weight.

$$\overline{M}_n(calc) = \frac{Monomer\ consumed}{Chains\ initiated} \approx \frac{[M]_0 - [M]_t}{[RAFT\ agent]} m_M \qquad (1)$$



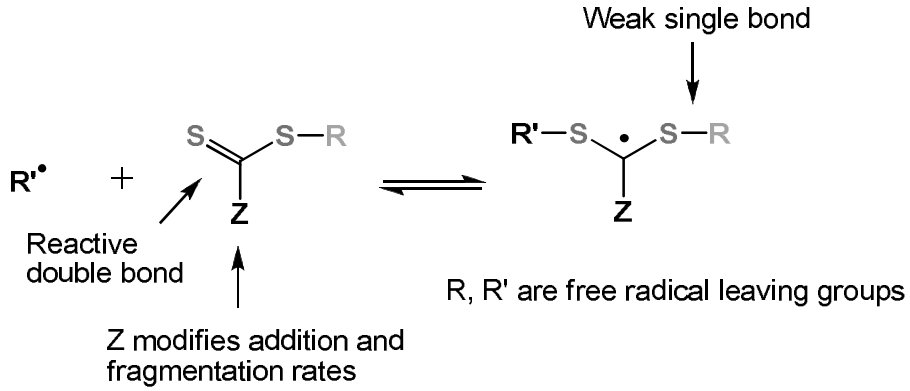
Scheme 14. Overall reaction in RAFT polymerization.[64]

- Narrow molecular weight distributions can be achieved.
- Blocks, stars and complex molecular architectures are accessible.

A wide variety of thiocarbonylthio RAFT agents have been reported.[64,79-82] These include certain trithiocarbonates, xanthates, dithiocarbamates and other compounds. The effectiveness

of the RAFT agent depends on the monomer being polymerized. Further, the properties of the free radical leaving group R and the group Z which can be chosen to activate or deactivate the thiocarbonyl double bond and to modify the stability of the intermediate radicals are crucial for a successful polymerization (see scheme 15).[81] For an efficient RAFT polymerization[64]

- the RAFT agents should have a reactive C=S double bond (high k_{add}),
- the intermediate radicals should fragment rapidly (high k_{β}, weak S-R bond) and no side reactions should occur,
- the intermediate should partition in favor of products ($k_{\beta} \geq k_{-add}$) and
- the expelled radicals should efficiently reinitiate polymerization.



Scheme 15: Structural features of thiocarbonylthio RAFT agent and the intermediate formed on radical addition.[64]

The RAFT polymerization in combination of functional polymers has been demonstrated as a powerful tool for the synthesis of complex polymers. The use of polymeric activated ester will be discussed in the next part of the dissertation.

Polymeric activated ester

The synthesis of functional polymers with defined molecular weight, defined composition, and defined architecture are still a challenge in polymer science. Functional polymers can be obtained from polymerization of suitable protected monomers. However, the additional deprotection step may not necessarily proceed completely. The direct polymerization of monomers bearing functional groups is clearly a more favored strategy.

The traditional living techniques, such as the anionic polymerization only offer very limited possibilities for the direct polymerization of functional monomers. This situation has changed with the development of controlled ("living") radical polymerization techniques. These polymerization methods showed a higher tolerance towards functional groups.[65-68] In spite of these advances, there is still a broad range of functionalities that cannot be introduced into a polymer by direct polymerization. Such functional groups may either completely prevent a controlled polymerization or may participate in side reactions. As a consequence to this, the control over the polymerization gets lost. Polymer analogous reaction[83] (also called post-polymerization modification[84]), is an attractive method for the synthesis of functional polymers that can overcome the limited functional group tolerance of many controlled "living" polymerization techniques.

The synthesis of functional polymers through a polymer analogous reaction is divided into two steps. Firstly, the polymerization of monomers with functional groups that are inert towards the polymerization conditions is performed. In the next step, the functional groups arranged at the polymer can quantitatively be converted in a subsequent reaction into a broad range of other functional groups (see figure 10).

Consequently, polymer analogous reaction allows access to functional polymers that cannot be prepared by direct polymerization of the corresponding functional monomer. This strategy is also highly attractive versatile for combinatorial materials discovery.[83,84] Activated esters have been established as very useful derivates for a covalent linking between amines and carboxylic acids resulting in stable amide bonds. The activated esters have found a broad application in peptide chemistry over many years.[83] Polymers bearing activated ester groups as a functional precursor have been reported firstly by Ringsdorf[85] and Ferruti[86] in 1972. In recent years, activated esters polymers have found broad applications in polymer chemistry as well as in material- and life science.

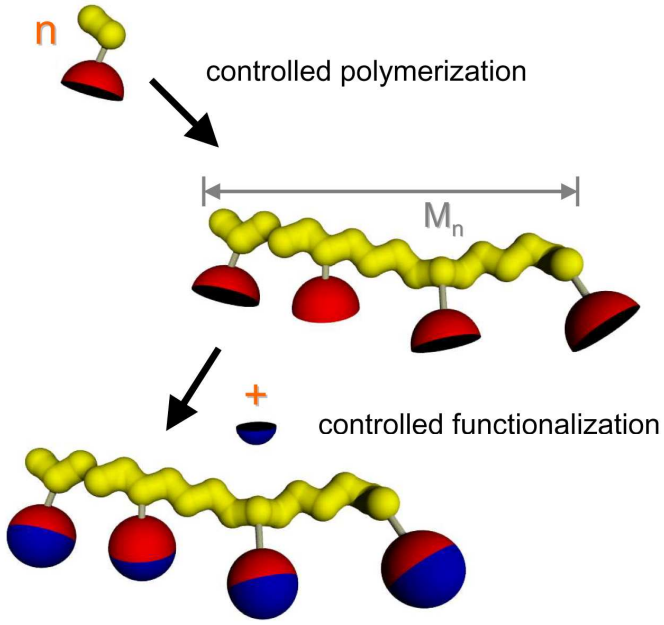
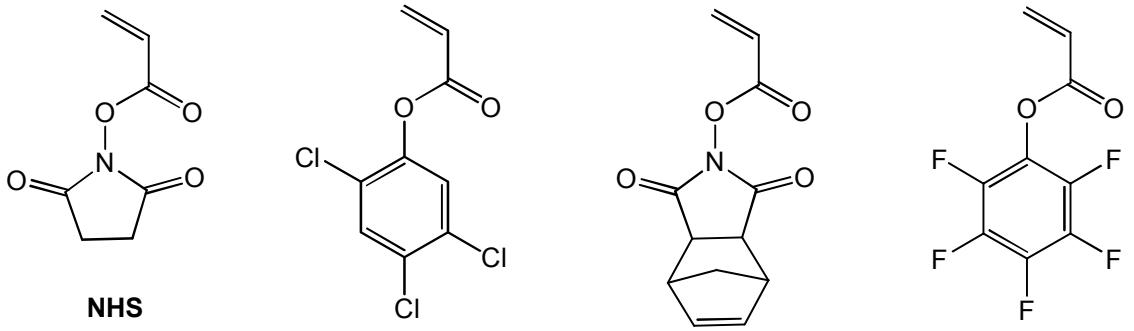


Figure 10: Schematic principle of a controlled polymerization of activated ester monomers followed by a controlled functionalization via a polymer analogous reaction.

The monomers *N*-hydroxy succinimide (NHS) acrylate (also called *N*-acryloxysuccinimide), and its methacrylate analogous, found extensive use for the synthesis of macromolecular precursors (see scheme 16).[83] These polymers, named conventionally poly(*N*-hydroxy succinimide acrylate) (PNHSA) and poly(*N*-hydroxy succinimide methacrylate) (PNHSMA) can be converted with amines in a polymer analogous reaction under very mild conditions into functionalized polyacrylamide or polymethacrylamide derivates, respectively.[87-90] However, a notable disadvantage of these two polymers is their limited solubility: PNHSA and PNHSMA are only soluble in DMF and DMSO.

The solubility of activated ester-based polymers can be improved by either copolymerization[91] or by replacing the NHS ester group with other activating groups, such as 2,4,5-trichlorophenol ester (TCP),[92] *endo*-*N*-hydroxy-5-norbornene-2,3-dicarboxyimide (NorbNHS),[92] or pentafluorophenol (PFP) ester groups (see scheme 16).[94-99] One of the advantages of poly(pentafluorophenyl acrylates) (PPFPA) and poly(pentafluorophenyl methacrylates) (PPFPMA) is their excellent solubility in common organic solvents.[83] Further, by using ^{19}F NMR spectroscopy, it is possible to monitor the reaction of the PFP ester groups. The conversion of PPFPA and PPFPMA activated esters with amines is usually quantitative and without any side reactions. The synthetic procedure occurs under mild conditions, such as stirring at room temperature.



Scheme 16: Chemical structures of various activated ester monomers.

Consequently, the reaction of activated esters with amines can be regarded as a prime example of reactions classified as ”click” reactions.[100] However, in contrast to the 1,3-dipolar cycloadditions, the reaction of activated esters with amines has the advantage to proceed without the auxiliary usage of a metal catalyst.
Following the advantages of activated ester chemistry in combination with RAFT polymerization, these techniques have been pointed out as the first choice method for the synthesis of thermoresponsive polymers bearing different functionalities.

As already mentioned above, cloud point measurements, also called turbidimetry, is one of the most common methods to determine the LCST of a thermoresponsive polymer in solution.[6,8] In the next few lines, the principle of a cloud point measurement will be presented shortly.

Cloud point measurements

In a typical cloud point measurement, the transmission intensity τ of a light beam that passes through a sample cell, i. e. the transmittance, is recorded versus the temperature of the polymer solution (see figure 11). Below the LCST, the polymer solution is totally clear and full transmittance of the incident light is observed. In the range of the LCST, the polymer starts to precipitate from solution due to transition from coil to a collapsed structure. As a consequence, the solution becomes turbid and the optical transmittance is decreased to a minimum. Normally, the phase transition is sharp and occurs within a temperature range of 1°C.[6,8] The LCST is therefore defined as the temperature in the diagram at which a transmission of 50% is observed.[8]

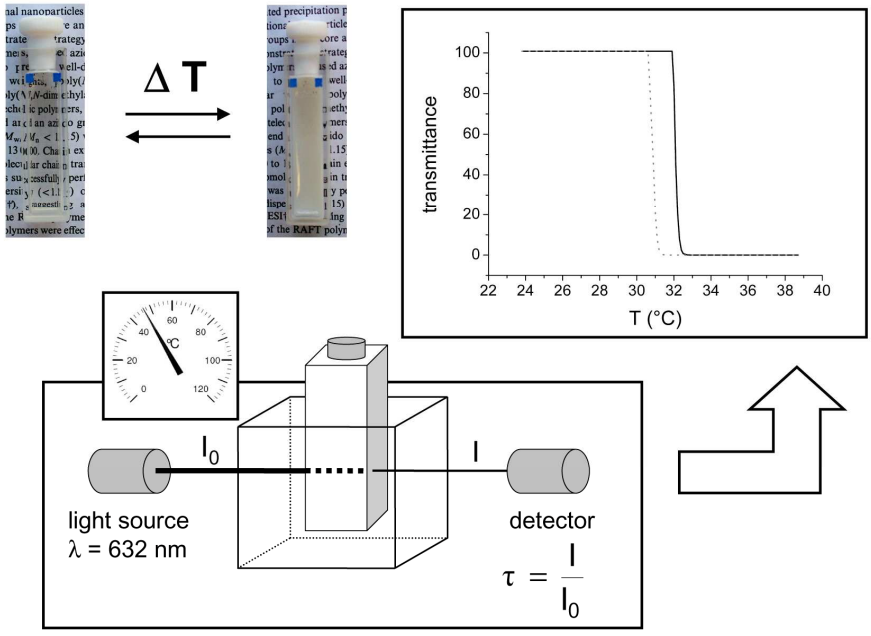


Figure 11. Schematic principle of a cloud point measurement to determine the LCST of polymer solutions.

Dynamic light scattering has been established as one of the most important methods to investigate the self-assembly behavior of functional block copolymers in solution. Within the next part of this dissertation, the theoretical background and the schematic setup of a dynamic light scattering experiment will be discussed.

Dynamic light scattering

Dynamic light scattering (DLS) (also known as photon correlation spectroscopy) is a physical technique to determine the size distribution profile of small particles in suspension or polymers in solution.[101-103] It can be used as well to investigate the behavior of complex fluids such as concentrated polymer solutions. DLS in combination with transmission electron microscopy (TEM) is the method of choice to analyze the hydrodynamic radii R_H of a polymeric micelle.

When light hits small particles the light is scattered in all directions (Rayleigh scattering) so long as the particles are small compared to the wavelength (below 250 nm). If the light source is a laser, providing monochromatic and coherent light, one can observe a time-dependent fluctuation in the scattering intensity. These fluctuations are due to the fact, that small molecules in solutions are undergoing Brownian motion. As a consequence, the distance between the scatterers is constantly changing with time. The scattered light undergoes either constructive or destructive interference by the surrounding particles. Within this intensity fluctuation, an information is generated about the time scale of movement of the scattering particles. The dynamic information is derived from an autocorrelation of the intensity trace recorded during the experiment. The second order autocorrelation $g^2(q;\tau)$ curve is as follows in equation 2:

$$g^2(q;\tau) = \frac{\langle I(t) I(t+\tau) \rangle}{\langle I(t) \rangle^2} \qquad (2)$$

with a particular wave vector q, the delay time τ and the intensity I of the scattered light. At short time delays, the correlation is high because the particles do not have a chance to move to a great extent from their initial state. Consequently, the two signals are unchanged when compared after a very short time interval. As the time delays become longer, the correlation starts to decay exponentially to zero. That means, after a long time period has elapsed, there is no correlation left between the scattered intensity of the initial and final states. This exponential decay is related to the motion of the particles, specifically to the diffusion in solution. Numerical methods are used, to fit the autocorrelation function (see figure 12).

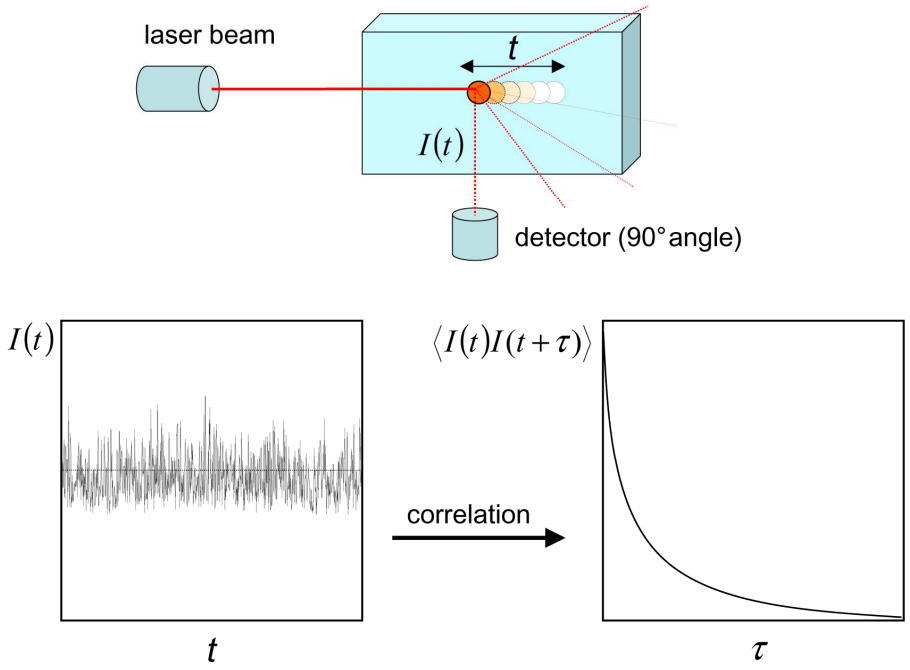


Figure 12. Schematic principle of a dynamic light scattering (DLS) setup using an autocorrelation function.

For monodisperse sample a single exponential decay can be observed. The Siegert equation relates the second order autocorrelation function with the first order autocorrelation function $g^1(q;\tau)$ as follows in equation 3:

$$g^2(q;\tau) = 1+\beta\left[g^1(q;\tau)\right]^2 \qquad (3)$$

where the parameter β is a correction factor that is depending on the geometry and the alignment of the laser beam in the light scattering setup. It is approximately equal to the inverse of the number of speckles. The most important use of the autocorrelation function is its use for size determination of particles.

Once the autocorrelation data have been generated, different mathematical approaches can be employed to determine size information of the scattering particles. The analysis is facilitated when an interaction of the scattering particles through collisions or electrostatic forces between ions can be excluded. Particle-particle collisions can be suppressed by dilution, whereas charge effects are reduced by the use of salts to collapse the electrical double layer.

The simplest approach is to treat the first order autocorrelation function as a single exponential decay (see equation 4). This is for a monodisperse population

$$g(q;\tau) = \exp(-\Gamma\tau) \qquad (4)$$

$$\text{with } \Gamma = q^2 D_t \qquad (5)$$

where Γ is the decay rate (see equation 5). The translational diffusion coefficient D_t from may be derived at a single angle or at a range of angles depending on the wave vector q

$$q = \frac{4\pi n_0}{\lambda}\sin\left(\frac{\theta}{2}\right) \qquad (6)$$

where λ is the incident laser wavelength, n_0 is the refractive index of the sample and θ is angle at which the detector is located with respect to the sample cell (see equation 6). D_t is often used to calculate the hydrodynamic radius R_H of a spherical particle through the Stokes-Einstein equation

$$D_t = \frac{k_B T}{6\pi\eta R_H} \qquad (7)$$

where k_B is the Boltzmann constant, T is the absolute temperature and η is the viscosity (equation 7).

It is important to note that the size determined by DLS is the size of a sphere that moves in the same manner as the scatterer. If the scatterer is a random coiled polymer, the determined size is not the same as the radius of gyration determined by static light scattering (SLS). It is also useful to point out that the obtained size will include any other molecules or solvent molecules that are surrounding the scattering particle.

References

(1) Lipowsky, R.; Sackman, E. *Handbook of Biological Physics* **1995**, *Vol. 1*, Elsevier Science B.V., ISBN: 9780444819758.

(2) Rodriguez-Hernandez, J.; Checot, F.; Gnanou, Y.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691-724.

(3) Lazzari, M.; Liu, G.; Lecommandoux, S. *Block Copolymers in Nanoscience* **2006**, Wiley-VCH, ISBN: 9783527313099.

(4) Gohy, J.-F. *Adv. Polym. Sci.* **2005**, *190*, 65-136.

(5) Riess, G. *Prog. Polym. Sci.* **2003**, *28*, 1107-1170.

(6) Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B. *Prog. Polym. Sci.* **2007**, *32*, 1275-1343.

(7) Rapoport, N. *Prog. Polym. Sci.* **2007**, *32*, 962-990.

(8) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163-249.

(9) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173-1222.

(10) Plate, N. A.; Lebedeva, T. L.; Valuev, L. I. *Polym. J.* **1999**, *31*, 21-27.

(11) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2001**, *34*, 1391-1399.

(12) Idziak, I.; Avoce, D.; Lessard, D.; Gravel, D.; Zhu, X. X. *Macromolecules* **1999**, *32*, 1260-1263.

(13) Meeussen, F.; Nies, E.; Berghmans, H.; Verbrugghe, S.; Goethals, E.; Du Prez, F. *Polymer* **2000**, *41*, 8597–8602.

(14) Uyama, H.; Kobayashi, S. *Chem. Lett.* **1992**, *21*, 1643–1646.

(15) Saito, S.; Otsuka, T. *J. Colloid Interface Sci.* **1967**, *25*, 531–536.

(16) Lutz, J.-F. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 3459-3470.

(17) Lutz, J.-F.; Akdemir, Ö.; Hoth, A. *J. Am. Chem. Soc.* **2006**, *128*, 13046-13047.

(18) Dai, S.; Ravi, P.; Tam, K. C. *Soft Matter* **2009**, *5*, 2513-2533.

(19) Zhao, Y. *J. Mater. Chem.* **2009**, *19*, 4887-4895.

(20) Ercole, F.; Davis, T. P.; Evans; R. A. *Polym. Chem.* **2010**, *1*, 37-54.

(21) Jiang, J.; Tong, X.; Morris, D.; Zhao, Y. *Macromolecules* **2006**, *39*, 4633-4640.

(22) Jiang, J.; Tong, X.; Zhao, Y. *J. Am. Chem. Soc.* **2005**, *127*, 8290-8291.

(23) Babin, J.; Pelletier, M.; Lepage, M.; Allard, J.-F.; Morris, D.; Zhao, Y. *Angew. Chem. Int. Ed.* **2009**, *48*, 3329-3332.

(24) Muraoka, T.; Cui, H.; Stupp, S. I. *J. Am. Chem. Soc.* **2008**, *130*, 2946-2947.

(25) Palmer, L.C.; Stupp, S. I. *Acc. Chem. Res.* **2008**, *41*, 1647-1684.

(26) Wang, G.; Tong, X.; Zhao, Y. *Macromolecules* **2004**, *37*, 8911-8917.

(27) Tong, X.; Wang, G.; Soldera, A.; Zhao, Y. *J. Phys. Chem. B.* **2005**, *109*, 20281-20287.

(28) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(29) Sin, S. L.; Gan, L. H.; Hu, X.; Tam, K. C.; Gan, Y. Y. *Macromolecules* **2005**, *38*, 3943-3948.

(30) Su., W.; Luo, Y.; Yan, Q.; Wu, S.; Han, K.; Zhang, Q.; Gu, Y.; Li, Y. *Macromol. Rapid Commun.* **2007**, *28*, 1251-1256.

(31) Wang., D.; Ye, G.; Wang, X. *Macromol. Rapid Commun.* **2007**, *28*, 2237-2243.

(32) Han, K.; Su, W.; Zhong, M.; Yan, Q.; Luo, Y.; Zhang, Q.; Li, Y. *Macromol. Rapid Commun.* **2008**, *29*, 1866-1870.

(33) Lin, L.; Yan, Z.; Gu, J.; Zhang, Y.; Feng, Z.; Yu, Y. *Macromol. Rapid. Commun.* **2009**, *30*, 1089-1093.

(34) Wang, D.; Liu, J.; Ye, G.; Wang, X. *Polymer* **2009**, *50*, 418-427.

(35) Kungwatchakun, D.; Irie, M. *Makromol. Chem. Rapid. Commun.* **1988**, *9*, 243-246.

(36) Kroeger, R.; Menzel, H.; Hallensleben, M. L. *Macromol. Chem. Phys.* **1994**, *195*, 2291-2298.

(37) Akiyama, H.; Tamaoki, N. *J. Polym. Sci. A. Polym. Chem.* **2004**, *42*, 5200-5214.

(38) Akiyama, H.; Tamaoki, N. *Macromolecules* **2007**, *40*, 5129-5132.

(39) Chunhua, L.; Fang, Z.; Zhaohui, Z.; Yuxing, P. *J. Appl. Polym. Sci.* **2007**, *107*, 2118-2125.

(40) Shimoboji, T.; Larenas, E.; Fowler, T.; Hoffman, A. S.; Stayton, P. S. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 16592-156596.

(41) Ravi, P.; Sin, S. L.; Gan, L. H.; Gan, Y. Y.; Tam, K. C.; Xia, X. L.; Hu, X. *Polymer* **2005**, *46*, 137-146.

(42) Jiang, X.; Lavender, C. A.; Woodcock, J. W.; Zhao, B. *Macromolecules* **2008**, *41*, 2632-2643.

(43) Crano, J. C.; Guglielmetti, R. J. *Organic Photochromic and Thermochromic Compounds* **2002**, *Vol. 1*, Kluwer Academic Publishers, ISBN: 0306458829.

(44) Yager, K. G.; Barrett, C. J. *J. Photochem. Photobiol. A. Chem.* **2006**, *182*, 250-261.

(45) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915-1925.

(46) Ichimura, K. *Chem. Rev.* **2000**, *100*, 1847-1873.

(47) Natansohn, A.; Rochon, P. *Chem. Rev*. **2002**, *102*, 4139-4175.

(48) Yu, Y.; Nakano, M.; Ikeda, T. *Nature* **2003**, *425*, 145.

(49) Nobel, A. *Annalen der Chemie und Pharmacie* **1856**, *98*, 253-256.

(50) Bigelow, H. E.; Robinson, D. B. *Org. Synth. Coll.* **1955**, *3*, 103-104.

(51) Brown, G. H. *Photochromism* **1971**, Wiley-Interscience New York, ISBN: 0471928941.

(52) Dürr, H.; Bouas-Laurent, H. *Photochromism: Molecules and Systems* **1990**, Elsevier Amsterdam, ISBN: 0444874321.

(53) Stobbe, H. *Berichte* **190**7, *40*, 3372-3382.

(54) Heller, H. G.; Elliot, C. C.; Koh, K.; Al-Shihry, S.; Whittall, J. *Spec. Publ.-R. Soc. Chem.* **1993**, *125*, 156-168.

(55) Anderson, D. G.; Wettermark, G. *J. Am. Chem. Soc.* **1965**, *87*, 1433-1438.

(56) Mitra, S.; Tamai, N. *Phys. Chem. Chem. Phys.* **2003**, *5*, 4647-4652.

(57) Barbara, P. F.; Rentzepis, P. M.; Brus, L. E. *J. Am. Chem. Soc.* **1980**, *102*, 2786-2791.

(58) Thompson, B. C.; Abboud, K. A.; Reynolds, J. R.; Nakatani, K.; Audebert, P. *New J. Chem.* **2005**, *29*, 1128-1134.

(59) Ohshima, A.; Momotake, A.; Arai, T. *Chem. Lett.* **2005**, *34*, 1288-1289.

(60) Suzuki, T.; Kaneko, Y.; Arai, T. *Chem. Lett.* **2000**, *29*, 756-757.

(61) Moad, G.; Solomon, D. H. *The Chemistry of Radical Polymerization* **2006**, *2nd Ed.*, Elsevier Oxford, ISBN: 0080442889.

(62) Davis, T. P.; Matyjaszewski, K. *Handbook of Radical Polymerization* **2002**, John Wiley & Sons Hoboken, ISBN: 047139274X.

(63) Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* **2007**, *32*, 93-146.

(64) Barner-Kowollik, C. *Handbook of RAFT Polymerization* **2008**, Wiley-VCH Weinheim, ISBN: 9783527319244.

(65) Hawker, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 11185-11186.

(66) Wang, J.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614-5615.

(67) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.

(68) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559-5562.

(69) Solomon, D. H. *J. Polym. Sci. A. Polym. Chem.* **2005**, *43*, 5748-5764.

(70) Chong, Y. K.; Ercole, F.; Moad, G.; Rizzardo, E.; Thang, S. H.; Anderson, A. G. *Macromolecules* **1999**, *32*, 6895-6903.

(71) Moad, G.; Rizzardo, E. *Macromolecules* **1995**, *28*, 8722-8728.

(72) Harker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661-3688.

(73) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689-3745.

(74) Cacioli, P.; Hawthorne, D. G.; Laslett, R. L.; Rizzardo, E. *J. Macromol. Sci. A. Chem.* **1986**, *23*, 839-852.

(75) Krstina, J.; Moad, G.; Rizzardo, E.; Winzor, C. L.; Berge, C. T.; Fryd, M. *Macromolecules* **1995**, *28*, 5381-5385.

(76) Delduc, P.; Tailhan, C.; Zard, S. Z. *Chem. Commun.* **1988**, *4*, 308-310.

(77) Zard, S. Z. *Angew. Chem. Int. Ed.* **1997**, *36*, 672-685.

(78) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379-410.

(79) Moad, G.; Chiefari, J.; Krstina, J.; Postma, A.; Mayadunne, R. T. A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* **2000**, *49*, 993-1001.

(80) Thang, S. H.; Chong, Y. K.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E. *Tetrahedron Lett.* **1999**, *40*, 2435-2438.

(81) Chong, Y. K.; Krstina, J.; Le, T. P. T.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2003**, *36*, 2256-2272.

(82) Roth, P. J.; Wiss, K. T.; Zentel, R.; Theato, P. *Macromolecules* **2008**, *41*, 8513-8519.

(83) Theato, P. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 6677-6687.

(84) Gauthier, M. A.; Gibson, M. I.; Klok, H.-A. *Angew. Chem. Int. Ed.* **2009**, *48*, 48-58.

(85) Batz, H. G.; Franzmann, G.; Ringsdorf, H. *Angew. Chem. Int. Ed.* **1972**, *11*, 1103-1104.

(86) Ferruti, A.; Bettelli, A.; Fere, A. *Polymer* **1972**, *13*, 462-464.

(87) Cline, G. W.; Hanna, S. B. *J. Am. Chem. Soc.* **1987**, *109*, 3087-3091.

(88) Cline, G. W.; Hanna, S. B. *J. Org. Chem.* **1988**, *53*, 3583-3586.

(89) Bergbreiter, D. E.; Hughes, R.; Besinaiz, J.; Li, C.; Osburn, P. L. *J. Am. Chem. Soc.* **2003**, *125*, 8244-8249.

(90) Wong, S. Y.; Putnam, D. *Bioconjugate Chem.* **2007**, *18*, 970–982.

(91) Favier, A.; D'Agosto, F.; Charreyre, M.-T.; Pichot, C. *Polymer* **2004**, *45*, 7821-7830.

(92) Theato, P.; Kim, J.-U.; Lee, J.-C. *Macromolecules* **2004**, *37*, 5475-5478.

(93) Eberhardt, M.; Mruk, R.; Zentel, R.; Theato, P. *Eur. Polym. J.* **2005**, *41*, 1569–1575.

(94) Eberhardt, M.; Theato, P. *Macromol. Rapid Commun.* **2005**, *26*, 1488–1493.

(95) Vogel, N.; Theato, P. *Macromol. Symp.* **2007**, *249/250*, 383-391.

(96) Barz, M.; Tarantola, M.; Fischer, K.; Schmidt, M.; Luxenhofer, R.; Janshoff, A.; Theato, P.; Zentel, R. *Biomacromolecules* **2008**, *9*, 3114-3118.

(97) Kessler, D.; Theato, P. *Langmuir* **2009**, *25*, 14200-14206.

(98) Kessler, D.; Roth, P. J.; Theato, P. *Langmuir* **2009**, *25*, 10068-10076.

(99) Metz, N.; Theato, P. *Macromolecules* **2009**, *42*, 37-39.

(100) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

(101) Brown, W. *Dynamic Light Scattering: The Method and Some Applications* **1993**, Oxford University Press, ISBN: 0198539428.

(102) Schmitz, K. S. *An Introduction to Dynamic Light Scattering by Macromolecules* **1990**, Academic Press, ISBN: 0126272603.

(103) Schärtl, W. *Light Scattering from Polymer Solutions and Nanoparticle Dispersions* **2007**, Springer Berlin, ISBN: 3540719504.

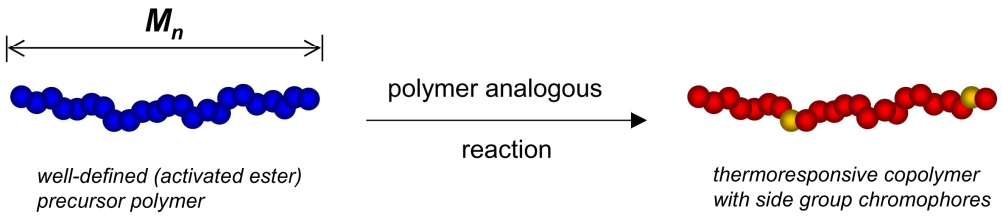
Scope of this work

The scope of this work is the synthesis and characterization of thermal- and lightresponsive copolymers and block copolymers. The synthesis of defined polymers that exhibit a LCST in water containing different amounts of various lightresponsive side- or end groups are to be developed utilizing modern polymerization methods, such as the reversible addition-fragmentation chain transfer (RAFT) polymerisation in combination with activated ester chemistry (see scheme 17). The key idea is to trigger the phase transition temperatures of these polymers by light-induced isomerization of the chromophores, which is usually accompanied with a local increase in polarity. Consequently, higher LCST values are expected upon irradiation, thus allowing a light-controlled solubility change within a certain temperature range.

However, the lightresponsive molecules attached onto a thermoresponsive polymer need to fulfil certain properties. As already mentioned, the isomerization of the molecules should be accompanied with a local increase in polarity. Therefore, the dyes might either be constructed in such a way, that they change their dipole moment or their chemical conformation upon irradiation with a specific wavelength. Consequently, a change from a non-polar structure into a higher polar or ionic structure would be desired. The isomerization should result in a maximum difference between the LCST of the non-irradiated and the irradiated species of the thermoresponsive polymer. Moreover, the light-induced reisomerization of the chromophores should be possible and the thermal-induced reisomerization should be negligible slow. As already mentioned, the isomerization of the dyes should only occur by using light of a specific wavelength and not by temperature or other physical or chemical stimuli. Further, the dyes may not interfere with water, neither in the non-irradiated nor in the irradiated state. Additionally, the chromophores should possess a photochemical high stability from bleaching effects and besides, they should not decompose.

Once having analyzed the properties of thermo- and lightresponsive copolymers, an extension of the present study would be the investigation of a stimuli controlled micellation process. Therefore, amphiphilic block copolymers with a thermo- and lightresponsive segment and solubility providing hydrophilic blocks need to be synthesized. The investigation of these materials is a certain challenge. These compounds might bear many interesting properties for several technical or biological applications.

Moreover, the synthesis of thermoresponsive polymers with different functional end groups is of great interest. As an extension on the study of the stimulated micellation, double thermal-responsive block copolymers with specific end groups are to be investigated in this dissertation.



Scheme 17. Synthetic concept for the synthesis of thermal- and lightresponsive copolymers via a polymer analogous reaction of well-defined precursor polymers.

Results

Thermoresponsive copolymers with photoswitchable side group chromophores

Thermoresponsive copolymers with azobenzene side groups

As discussed previously, the synthesis of well-defined and narrow distributed thermo- and lightresponsive polymers is still a challenge. Herein, the synthetic procedure of four different series of thermoresponsive polyacrylamide copolymers each bearing different amounts of photoswitchable azobenzene side groups was described. The synthesis was based on the polymer analogous reaction of well-defined PPFPA with different primary or secondary amines. All copolymers were designed to exhibit a LCST in aqueous solution, which was dependent on (i) the amount of incorporated azobenzene chromophores and (ii) the isomerization state of the azobenzene group. The cardinal idea was to shift the LCST of the copolymers up to higher values upon irradiation, due to the *trans* to *cis* isomerization of the azobenzene units, which is in general accompanied with a change of the dipole moment μ. In fact, higher LCST values were measured for each UV-irradiated copolymer solution in comparison to the non-irradiated copolymer solution. The light-controlled LCST increase was fully reversible due to the reisomerization of the chromophore under visible light exposure (see figure 13). However, the LCST shifts did not show any significant dependency on the amount of azobenzene chromophore. For some reasons, in one copolymer series the LCST shifts increased with increasing amount of azobenzene side groups while for another copolymer series the LCST shift remained almost constant. A maximum LCST difference of 7°C was found for a copolymer of poly(*N*,*N*-dimethylacrylamide) containing 8.5 mol% of azobenzene groups. Within this temperature range, a reversible solubility change by irradiation with UV-light was possible, which was successfully proved by a kinetic transmission measurement of a polymer solution at a fixed temperature above the LCST.

Publication 1: Jochum, F. D.; Theato, P. *Polymer* **2009**, *50*, 3079-3085.

Thermoresponsive copolymers with salicylideneaniline side groups

Herein, two different series of thermoresponsive polyacrylamides containing different amounts of salicylideneaniline moieties as lightresponsive groups were investigated. Like

azobenzene, salicylideneaniline chromophores are well known to undergo reversible isomerization upon irradiation with UV-light. The isomerization goes along with a *keto-enol* tautomerization. For the synthesis of the copolymers, PPFPA was used again as a defined precursor polymer for the post-modification with amines. Both copolymer series exhibited a LCST in aqueous solution. The phase transition behavior of every polymer solution was investigated by turbidimetry. The LCST was dependent on the amount of hydrophobic salicylideneaniline moieties and their isomerization states. The irradiation of the copolymer solutions with UV-light was followed with by an irreversible increase of the LCST (see figure 13). A maximum LCST shift of 13°C was found for a poly(*N*-cyclopropylacrylamide) copolymer with 15 mol% of salicylideneaniline side groups. A further explanation for the high LCST differences might be a decomposition of the chromophore. However, this could be excluded in this study by several individual spectroscopy measurements.

Publication 2: Jochum, F. D.; Theato, P. *Macromolecules* **2009**, *42*, 5941-5945.

Thermoresponsive copolymers with fulgimide side groups

A series of PNIPAM based copolymers featuring a fulgimide as a lightresponsive side group chromophore was investigated. The synthesis of these materials was done by a polymer analogous reaction of PPFPA with primary amines. All PNIPAM based copolymers showed a LCST in aqueous solution. In contrast to azobenzene and salicylideneaniline bearing copolymers, the LCST was only tiny affected by incorporating the fulgimide chromophore. A photocyclization of the chromophore by irradiation of the solutions with UV-light was observed. This isomerization process of the fulgimide side groups was accompanied with a color change of the solutions from colorless into red. The closed form of the dye was thermally stable and had a halftime of 136 min for the visible reisomerization. However, the chromophore isomerization did not influence the LCST in contrast to the azobenzene or salicylideneaniline dyes incorporated into PNIPAM polymers (see figure 13). Following this principle, the solubility of these materials was only a function of the temperature. This led to the realization of a logic “NOT A” molecular gate for these copolymers, while the corresponding copolymers with azobenzene side groups would be an example for the molecular logic gate “A implies B”.

Publication 3: Jochum, F. D.; Forst, F. R..; Theato, P. *Macromol. Rapid Commun.* **2010**, *31*, 1456-1461.

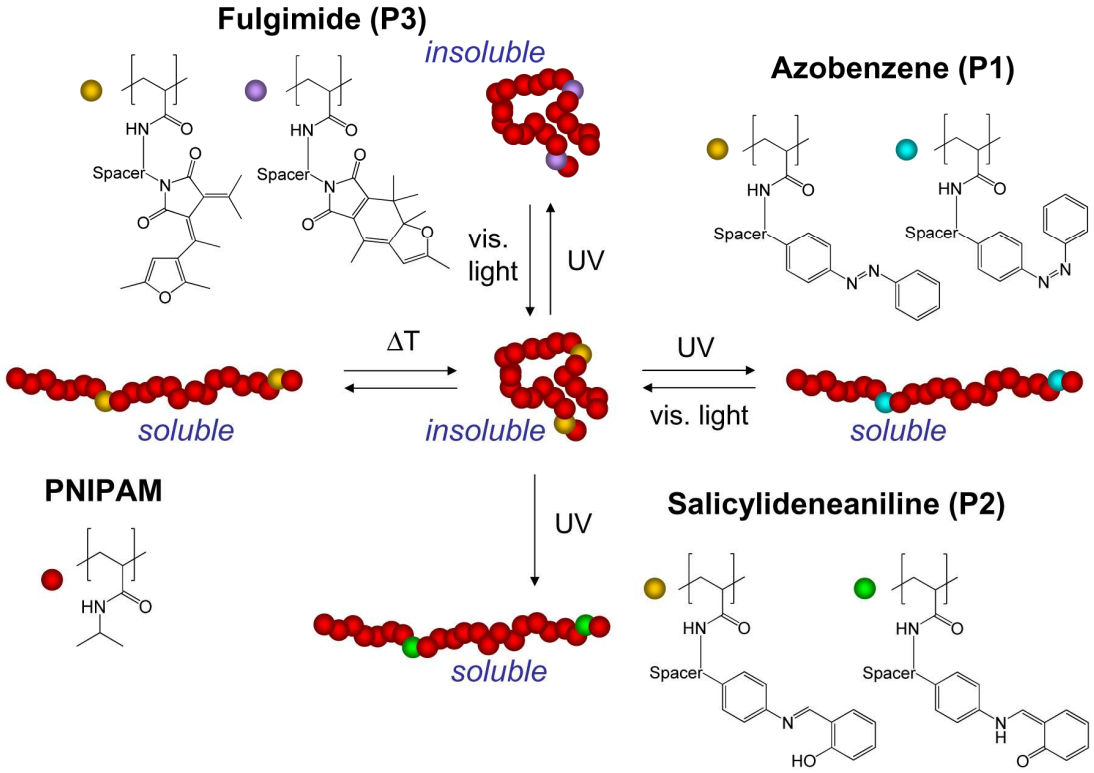


Figure 13. Overview of all reactions of thermal- and lightresponsive PNIPAM based copolymers with three different chromophores, respectively. Azobenzene bearing PNIPAM copolymers showed a reversible temperature- and light-induced solubility change within a certain temperature range (P1). For PNIPAM based copolymers with salicylideneaniline side groups an irreversible solubility change was found (P2), whereas fulgimide irradiated thermoresponsive copolymers did not influence the LCST at all (P3).

Thermoresponsive polymers with photoswitchable azobenzene end groups

In this project, thermo- and lightresponsive polymers based on poly(oligo(ethylene glycol) methyl ether methacrylate) P(OEGMA) with azobenzene end groups were synthesized. RAFT polymerization using a functional CTA containing a PFP ester was performed to polymerize oligo(ethylene glycol)methyl ether methacrylate (OEGMA, M_n ~ 300 g mol^{-1}) yielding end group functionalized, narrow distributed thermoresponsive polymers in two different chain length. These polymers possessed an activated ester PFP α-end group as well as a dithioester ω-terminus. The dithioester could quantitatively be removed by AIBN treatment or substituted with a PFP ester by using a modified activated ester diazo-compound. Consequently, a post-modification of the reactive end groups with amino-functionalized azobenzene was possible. The resulting P(OEGMA) polymers contained azobenzene either on the α-end group or on both (telechelic) ends of the polymer chain. The sample solutions in water showed a reversible thermo- and light-controlled phase transition (Figure 14).

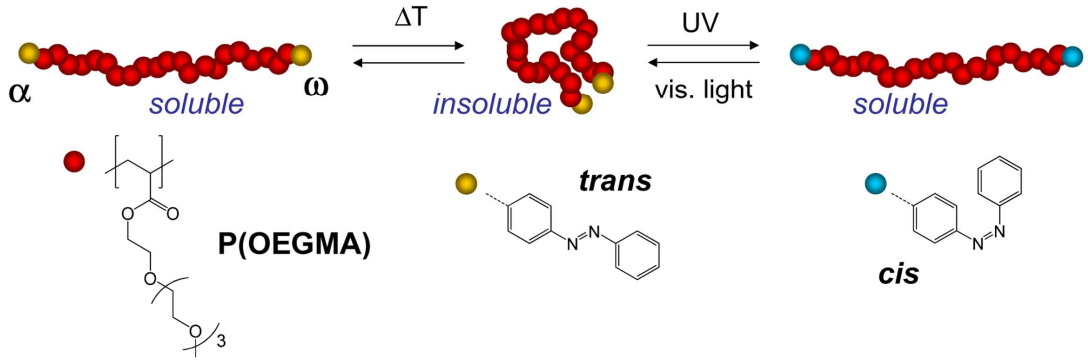


Figure 14. Overview of all reactions of thermal- and lightresponsive P(OEGMA) polymers with azobenzene end groups.

Similar to thermoresponsive copolymers with azobenzene side groups, higher LCST values were measured after irradiation with UV-light due to the *trans*-to-*cis* isomerization of the end group chromophores, respectively. In all cases, the isomerization process of the azobenzene end groups was fully reversible. The initial values for the phase transition temperature were measured after visible light exposure.

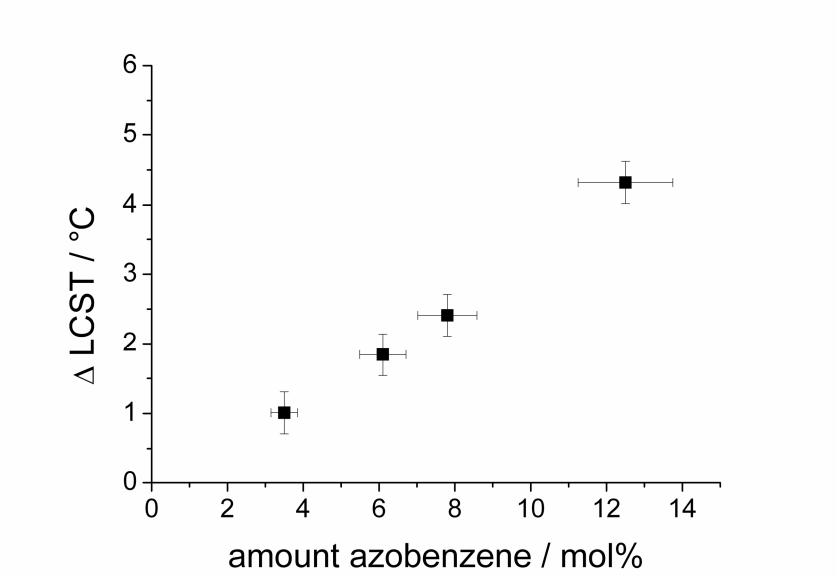


Figure 15. Linear increase of the LCST shifts of azobenzene-terminated P(OEGMA) polymers.

The LCST differences between irradiated and non-irradiated solutions increased linearly upon the ratio of azobenzene end groups up to a maximum of 4.3°C (see figure 15). Noteworthy, the chemical nature of the end group had a high influence on the LCST.

Publication 4: Jochum, F. D.; zur Borg, L.; Roth, P. J.; Theato, P. *Macromolecules* **2009**, *42*, 7854-7862.

Thermo- and lightresponsive micellation of azobenzene containing PEO-*b*-PNIPAM block copolymers

Once having investigated the temperature- and lightresponsive behavior of PNIPAM based copolymers with azobenzene moieties, these findings were used for dual responsive block copolymers (see figure 16). The key idea was to synthesize "smart materials" that self-assemble in water into micelles by external stimuli such as temperature and light. For that, block copolymers with a temperature- and lightresponsive block and a solubility providing block were synthesized. The dual-responsive block contained a PNIPAM copolymer with azobenzene side groups while poly(ethylene oxide) (PEO) was used as hydrophobic block segment. The synthesis was based on a polymer analogous reaction of a PEO-*b*-PPFPA precursor block copolymers with functional amines. PEO-*b*-PNIPAM with azobenzene side groups was capable to self-assemble into micellar structure at higher temperatures. Above the LCST, the PNIPAM block became insoluble in water and formed the hydrophobic core of a micellar structure, while the PEO block formed the hydrophilic corona. The temperature controlled reversible micellation process was monitored by different individual techniques such a DLS, turbidimetry, or temperature dependent fluorescence and NMR measurements. The polymeric micelles were capable to encapsulate hydrophobic coumarin 102 dye into the core. Further, light-controlled disruption of the aggregates by using UV-light at temperatures above the LCST could be demonstrated successfully.

Publication 5: Jochum, F. D.; Theato, P. *Chem. Comm.* **2010**, *46*, 6717-6719.

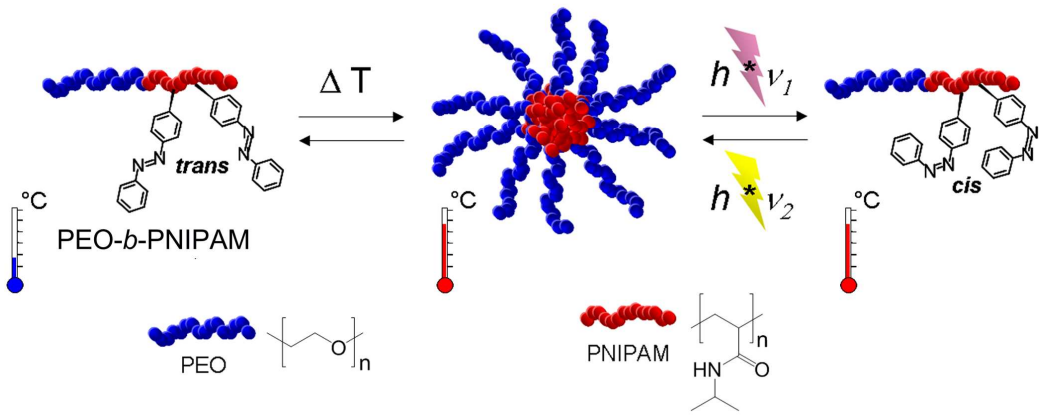


Figure 16. Schematic principle of thermo- and lightresponsive micellation of functional PEO-b-PNIPAM block copolymers with azobenzene side groups.

Double thermoresponsive block copolymers with biotin end group

As an extension of the previous work, the stimuli micellation of functional double thermoresponsive block copolymers with bio targeting end groups was investigated. In this study, poly[(oligo(ethylene glycol) monomethyl ether methacrylate)-*block*-(*N*-isopropyl methacrylamide)] (POEGMA-*b*-PNIPMAM) block copolymer with a biotin end group on the PNIPMAM block was synthesized as a model system for temperature controlled polymer immobilization. The synthesis was based on RAFT polymerization followed by post-modification reaction of an activated ester precursor block and an exchange of the dithioester end group within one step. The stimuli responsive behavior of POEGMA-*b*-PNIPMAM was analyzed by NMR, differential scanning calorimetry (DSC), dynamic light scattering (DLS) and turbidimetry measurements. The double thermoresponsive POEGMA-*b*-PNIPMAM showed a temperature dependent multi-stage assembly behavior as it was completely soluble in water at temperatures below the LCST of both blocks, formed micellar structures above the LCST of PNIPMAM but below the LCST of POEGMA or precipitated from solution above the LCST of both blocks. At room temperature, the polymer could be immobilized onto a streptavidin surface via its biotin end group, as shown in surface plasmon resonance (SPR) experiments. At 50°C, at which the block copolymer formed micelles trapping the biotin target within the PNIPMAM core, no immobilization was observed, showing that the biological binding ability of the model could be controlled via external stimuli (see figure 17).

Publication 6: Jochum, F. D.; Roth, P. J.; Kessler, D.; Theato, P. *Biomacromolecules* **2010**, *11*, 2432-2439.

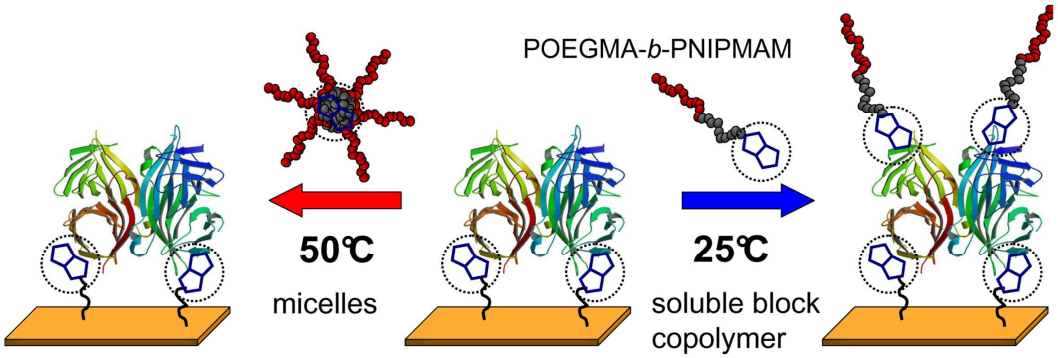


Figure 17. Schematic principle of a temperature-controlled immobilization of biotin end-capped double thermoresponsive POEGMA-b-PNIPMAM block copolymers.

Summary and Conclusion

In summary, thermoresponsive polyacrylamides with various amounts of different photoswitchable side groups, i. e. azobenzene, salicylideneaniline and fulgimide were successfully prepared. As such, in a first step three different chromophores with an amine functionality were synthesized. The synthesis of the stimuli-responsive materials was based on the RAFT polymerization of activated ester acrylates followed by a polymer analogous reaction with different amines. The procedure has been designed to allow the synthesis of well-defined materials with functional groups. All copolymers prepared in this way showed a LCST in aqueous solution. The LCST was in general decreased by increasing the amount of hydrophobic dye incorporated into the thermoresponsive polymer. However, in the case of the fulgimide, the LCST was hardly affected by the chromophore. For azobenzene containing PNIPAM polymers and analogues, higher LCST values were measured after irradiation of the polymer sample solutions with UV-light (Δ $LCST_{max}$ = 7.3°C). A reversible light-induced solubility change within a certain temperature range was possible. In contrast to this, irradiated samples of salicylideneaniline containing thermoresponsive copolymers showed an irreversible increase in the LCST (Δ $LCST_{max}$ = 13.0°C). Fulgimide chromophores did not influence the LCST of PNIPAM based copolymers after UV-light exposure.

Similar to the thermoresponsive polyacrylamides with azobenzene side groups, poly(oligo(ethylene glycol) methyl ether methacrylate) [P(OEGMA)] polymers with azobenzene end groups showed a LCST shift upon UV-irradiation. These polymers were synthesized by RAFT polymerization using a functional chain transfer agent (CTA). For this, *PFP*-CTA was used as a RAFT-agent for end group functionalization of (thermoresponsive) polymers. In contrast to the statistically arranged copolymers with azobenzene side groups, P(OEGMA) polymers with terminal azobenzene showed a linear increase of the LCST shifts with increasing amount of chromophore (Δ $LCST_{max}$ = 4.3°C). Noteworthy, the chemical nature of the end group exhibited a strong influence on the LCST in the case of short thermoresponsive P(OEGMA) polymers.

The investigation on temperature- and lightresponsive polymers was transferred onto block copolymers capable to self-assemble into polymeric micelles. Therefore, PEO-*b*-PNIPAM block copolymers with azobenzene moieties were synthesized successfully. These polymers showed a “smart” behavior in aqueous solution, as the reversible formation and disruption of

the micelles could either be controlled by temperature or using light as a stimulus. The usefulness of these materials was demonstrated by encapsulation of a hydrophobic dye in the core of the micelle. Such materials might have a great potential as a model system for several technical or biological applications.

Finally, double thermoresponsive block copolymers forming micellar structures in a certain temperature range with functional end groups could successfully be synthesized. These “smart materials” based on POEGMA-*b*-PNIPMAM have been demonstrated to be very promising for a temperature selective immobilization on a protein surface. This might be a suitable concept for further biological applications.

Concluding, different thermoresponsive copolymers and block copolymers with lightresponsive moieties arranged along the backbone or located at the chain ends were successfully prepared and investigated. By controlling the nature of functional groups and their respective incorporation ratios, the LCST could be dialed in precisely. Further, the LCST of the polymers could be triggered by light. A light-controlled disruption of micellar structures could be shown for functional block copolymers. The importance of end groups of thermoresponsive polymers was demonstrated by a temperature-controlled protein-polymer binding of a terminal biotin-functionalized double thermoresponsive polymer. The synthetic approaches and the material properties presented here should be promising for further research and applications beyond this dissertation.

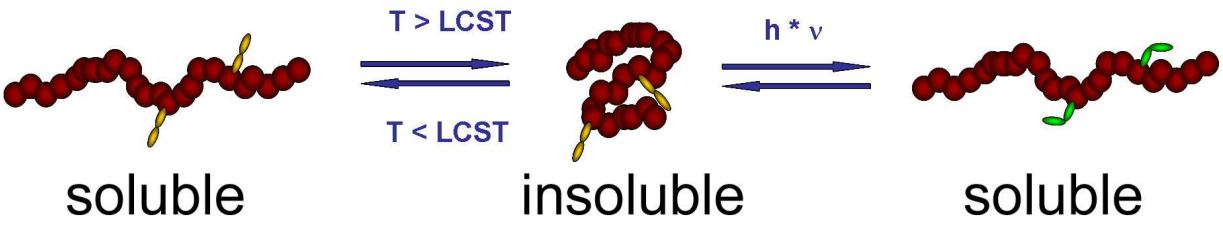
Publications

Temperature and Light Sensitive Copolymers Containing Azobenzene Moieties Prepared via a Polymer Analogous Reaction

*Florian D. Jochum, Patrick Theato**

ABSTRACT

Four different series of polyacrylamides containing different amounts of azobenzene moieties have been synthesized via a polymer analogous reaction of poly(pentafluorophenylacrylate) (PPFPA). All copolymers were designed to exhibit a lower critical solution temperature (LCST) in aqueous solution, which was dependent on (i) the amount of incorporated chromophoric azobenzene groups and (ii) the isomerization state of the respective azobenzene group. Higher LCST values were measured for UV-irradiated solutions of the copolymers in comparison to the non-irradiated copolymer solutions. A maximum difference in the LCST of up to 7 °C was found for the copolymer poly(*N*,*N*-dimethylacrylamide) containing 8.5 mol% of azobenzene groups. Within this temperature range, a reversible solubility change of the copolymer could be induced by irradiation with light.



Introduction

Some water-soluble polymers show an unusual behavior in aqueous solution, as these synthetic materials undergo a phase separation upon heating. In this context, especially polyacrylamide derivates, such as poly(*N*-isopropylacrylamide) (PNIPAM) found great interest, because PNIPAM features a sharp transition behavior in water in response to temperature changes.[1] This lower critical transition temperature (LCST) appears for PNIPAM in water at 32 °C.

Besides PNIPAM also other polymers exhibit such a temperature induced transition in water. Examples are poly(*N*-ethyl-*N*-methylacrylamide),[2] poly(*N*-cyclopropylacryl-amide)[3] or poly(*N*,*N*-diethylacrylamide).[4] Accordingly, such polymers featuring a LCST are expected to find application in many scientific areas, such as drug delivery[5] or the immobilization of enzymes and cells[6] because the LCST is close to body temperature.

The area of polymers responsive to a single stimulus has been extended to polymers, which show a responsive behavior to multiple stimuli. Other stimuli besides temperature can for example be pH,[7] ionic strength[8] or light.[9,10]

Of special importance seem polymers that are responsive to light and temperature and accordingly, there have been several reports on temperature-responsive polymers, for example those containing a light-responsive azobenzene moiety.[11-18] In all these reports, appropriate azobenzene containing monomers have been copolymerized with either *N*-isopropylacrylamide (NIPAM) or *N*,*N*-dimethylacrylamide (DMA) yielding light- and temperature-responsive copolymers. The LCST of these azobenzene containing copolymers depends not only on the amount of chromophoric groups but also on the degree of isomerisation of the azobenzene. Azobenzene groups are known to undergo reversible isomerisation from *trans*- to *cis*-configuration upon irradiation.[19,20] In the excited *cis*-configuration, the higher dipole moment leads to an increase of local polarity of the polymer chain, which causes an increase of the LCST.[21] And as a result, these copolymers can be precipitated upon irradiation with light within a certain temperature range.

As we could describe in previous publications, the polymer analogous reaction of activated ester polymers provides many synthetic advantages.[22-25] In combination with modern methods of controlled radical polymerization, like the RAFT polymerization, one can receive precisely defined reactive polymer structures.[26] This procedure enables the synthesis of polymeric architectures, which cannot be realized by the classical way of radical copolymerization.

PPFPA is known to react quantitatively with aliphatic amines yielding the respective polyacrylamide polymers and copolymers.[22] Additionally, this post modification of activated polymers has the advantage that the synthesis is totally independent from the copolymerization parameters of the respective acrylamide monomers. Furthermore, with this method one can even obtain copolymers in the case where the corresponding monomers would be impossible to be copolymerized.

In this manuscript, we report on the synthesis and characterization of four different series of thermo- and light-responsive polyacrylamides that use azobenzenes as the corresponding photochromic groups. In contrast to previous reports, all copolymers will be prepared via a polymer analogous reaction of activated ester precursor polymers on the basis of poly(pentafluorophenylacrylate) (PPFPA),[22] which will be synthesized by the reversible addition fragmentation chain transfer (RAFT)

polymerisation.[26] The LCST behavior of the resulting polyacrylamides with different amounts of azobenzenes will be investigated in detail.

Experimental Section

Materials. All chemicals and solvents were commercially available and used as received unless otherwise stated. Tetrahydrofurane (THF), 1,4-dioxane and diethyl ether were previous distilled over sodium. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at -7 °C. As dialysis membranes Spectra/Por 3 (MWCO 3500) were used. Benzyl dithiobenzoate was synthesized as a chain transfer agent for RAFT-polymerization as described in the literature. Briefly, dithiobenzoic acid magnesium salt was reacted with and the benzylbromid.[27]

Instrumentation. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz FT-NMR spectrometer in deuterated solvents. ^{19}F NMR spectra were recorded on a Bruker 400 MHz FT-NMR spectrometer. Chemical shifts (δ) were given in ppm relative to TMS. Gel permeation chromatography (GPC) was used to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards. GPC measurements were performed in THF as solvent and with the following parts: pump PU 1580, auto sampler AS 1555, UV-detector UV 1575, RI-detector RI 1530 from Jasco, and miniDAWN Tristar light scattering detector from Wyatt. Columns were used from MZ-Analysentechnik: MZ-Gel SDplus 102 Å, MZ-Gel SDplus 104 Å and MZ-Gel SDplus 106 Å. The elution diagrams were analyzed using the ASTRA 4.73.04 software from Wyatt Technology. Calibration was done using polystyrene standards. The flow rate was 1 mL/min at a temperature of 25 °C. UV/Vis spectra were recorded on a Shimadzu UV PC 2102 photospectrometer. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer using an ATR unit. FD-masses were measured on a MAT 95 Finnigan mass spectrometer. Cloud points were determined in Millipore water at a concentration of 20 mg/mL and were observed by optical transmittance of a light beam (λ = 632 nm) through a 1 cm sample quartz cell. The measurements were performed in a Jasco V-630 photospectrometer with a Jasco ETC-717 peltier element. The intensities of the transmitted light were recorded versus the temperature of the sample cell. The heating rate was 1 °C per minute. Irradiation of polymer samples was performed in a quartz cell with a diameter of 1 cm using an Oriel Instruments 500 W mercury lamp with a 365nm optical filter with an average output of 10 mW/cm^2. For the real-time photo-switch experiment an optical fiber from Oriel Instruments and a filter for visible light irradiation (λ>400 nm) were additionally used.

Pentafluorophenylacrylate (PFPA). 80 g (0.43 mol) pentafluorophenol and 52.5 g (0.52 mol) triethylamine (TEA) were dissolved in 500 mL dry diethyl ether and 47.2 g (0.52 mol) acryloyl chloride was added dropwise through a funnel under cooling with an ice bath. After stirring additional 2 hours at room temperature, the precipitated salt was removed by filtration. After evaporation of the solvent, the residue was filtered again and purified with column chromatography (column material: silica gel, solvent: petroleum ether). 75 g (0.32 mol – 74 %) of a colourless liquid were obtained. The pure PFPA was stored at -7 °C. ^{1}H NMR ($CDCl_3$): δ /ppm: 6.70 (d, 1H), 6.35 (dd, 1H), 6.16 (d, 1H); ^{13}C NMR ($CDCl_3$): δ /ppm: 161.5 (s), 142.8 (m), 141.2 (m), 139.5 (m), 137.9 (m), 136.2 (m), 134.9 (s), 125.1 (s); ^{19}F NMR (CDCl3): δ /ppm: -162.77 (d, 2F), -158.39 (t, 1F), -153.02 (d, 2F); FT-IR (ATR-mode): 1772 cm^{-1} (C=O reactive ester band), 1516 (C=C aromatic band)

Poly(pentafluorophenylacrylate) (PPFPA). In a typical RAFT polymerization a mixture of 20 g (0.084 mol) PFPA, 51.5 mg (2.11 * 10^{-4} mol) benzyl dithiobenzoate and 4.3 mg (2.62 * 10^{-5} mol) AIBN were placed into a Schlenk-flask. After addition of 50 mL dry dioxane, four freeze-pump-thaw cycles were performed to degas the solution. The flask was filled with argon, immersed in a preheated oil bath of 80 °C and stirred for 20 hours. After cooling down to room temperature, the polymer was isolated by precipitation in hexane. The crude polymer was dissolved in THF, precipitated again twice into hexane, centrifuged and finally dried in a vacuum oven at 40 °C. Yield 13 g (65 %) of a pink powder. ^{1}H NMR ($CDCl_3$): δ /ppm: 3.10 (br s), 2.51 (br s), 2.13 (br s); ^{13}C NMR ($CDCl_3$): δ /ppm: 169.28 (s), 142.24 (m), 138.93 (m), 135.77 (m), 40.11 (br s), 34.27 (br s); ^{19}F NMR ($CDCl_3$): δ /ppm: -162.28 (br s), -157.15 (br s), -153.56 (br s); FT-IR (ATR-mode): 1783 cm^{-1} (C=O reactive ester band), 1515 cm^{-1} (C=C aromatic band), 1090 cm^{-1} (C-O ester band)

Pentafluorophenyl-4-(2-phenyldiazenyl) benzoate. 2.5 g (0.011 mol) (4-(2-phenyldiazenyl)benzoic acid and 2.9 g (0.029 mol) TEA were dissolved in 50 mL THF and 7.7 g (0.028 mol) pentafluorophenyl trifluoracetate in 30 mL THF were added slowly through a dropping funnel. After stirring for 2 h at room temperature, 150 mL of dichloromethane were added and the solution was extracted twice with 30 mL of water. The organic layer was isolated and dried with sodium sulfate. After evaporation of most of the solvent, the final product was obtained by precipitation into hexane. After filtration and drying in vacuum at 40 °C, a red colored solid was obtained in 2.7 g yield (0.0069 mol – 63 %). ^{1}H NMR ($CDCl_3$): δ /ppm: 8.34 (d, 2H), 8.03 (d, 2H), 7.96 (m, 2H), 7.54 (m, 3H); FT-IR (ATR-mode): 1759 cm^{-1} (C=O reactive ester band), 1516 cm^{-1} (C=C aromatic band)

***N*-(2-aminoethyl)-4-(2-phenyldiazenyl) benzamide.** 2.3 g (0.038 mol) 1,2-diamino ethane were dissolved in 50 mL THF and 1.5 g (0.0038 mol) pentafluorophenyl 4-(2-phenyl-diazenyl)benzoate dissolved in 30 mL THF were added slowly at room temperature through a dropping funnel. After 3 h of stirring at room temperature, 120 mL of dichloromethane were added and the solution was washed four times with 30 mL water. The organic layer was isolated and dried with sodium sulfate. After complete evaporation of the solvent, 0.83 g (0.0031 mol - 82 %) of orange colored *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide were obtained. ^{1}H NMR (DMSO): δ /ppm: 8.63 (s, 1H), 8.06 (d, 2H), 7.93 (m, 4H), 7.60 (m, 3H), 3.30 (q, 2H), 2.72 (t, 2H); FT-IR (ATR-mode): 3292 cm^{-1} (primary amine band), 1634 cm^{-1} (C=O amide band I), 1540 cm^{-1} (C=O amide band II); MS (FD) m/z (%): 270.4 (0.39), 269.4 (9.89), 268.4 (100.00)

Synthesis of the temperature- and light sensitive copolymers P1. To three different solutions containing each 1.5 g of PPFPA and 2 mL TEA in 20 mL THF were added dropwise 16.9 mg (P1a: 6.30 * 10^{-5} mol), 50.7 mg (P1b: 1.89 * 10^{-4} mol) and 84.5 mg (P1c: 3.15 * 10^{-4} mol) of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide dissolved in 10 mL THF. The solutions were stirred for 3 hours under nitrogen atmosphere at room temperature. Afterwards, 2 mL of isopropylamine (0.023 mol) were added into each flask. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The orange colored residues were suspended in 10 mL of water and dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were twice dissolved in 5 mL of THF and precipitated into hexane. Usually, after centrifugation and drying in a vacuum oven at 40 °C, between 0.54 g and 0.65 g of yellow-orange polymers were obtained. ^{1}H NMR (MeOD): δ

/ppm: 8.00 (br s), 7.66 (br s), 3.99 (s), 2.13 (br s), 1.63 (br s), 1.19 (s); FT-IR (ATR-mode): 3302 cm^{-1} (amide N-H-valence), 1644 cm^{-1} (C=O amide band I), 1540 cm^{-1} (C=O amide band II)

Synthesis of the temperature- and light sensitive copolymers P2. To four different solutions containing each 1.5 g of PPFPA and 2 mL TEA in 20 mL THF were added dropwise 16.9 mg (P2a: 6.30 * 10^{-5} mol), 50.7 mg (P2b: 1.89 * 10^{-4} mol), 84.5 mg (P2c: 3.15 * 10^{-4} mol) and 135.2 mg (P2d: 5.04 * 10^{-4} mol) of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide dissolved in 10 mL THF. The solutions were stirred for 3 hours under nitrogen atmosphere at room temperature. Afterwards, 2 mL of cyclopropylamine (0.029 mol) were added into each flask. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The orange colored residues were suspended in 10 mL of water and dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were twice dissolved in 5 mL of methanol and precipitated into diethyl ether. Usually, after centrifugation and drying in a vacuum oven at 40 °C, between 0.49 g and 0.58 g of yellow-orange polymers were obtained. ^{1}H NMR (MeOD): δ /ppm: 8.01 (br s), 7.58 (br s), 2.72 (s), 2.07 (br s), 1.58 (br s), 0.66 (d); FT-IR (ATR-mode): 3291 cm^{-1} (amide N-H-valence), 1652 cm^{-1} (C=O amide band I), 1539 cm^{-1} (C=O amide band II)

Synthesis of the temperature- and light sensitive copolymers P3. To three different solutions containing each 1.5 g of PPFPA and 2 mL TEA in 20 mL THF were added dropwise 16.9 mg (P3a: 6.30 * 10^{-5} mol), 50.7 mg (P3b: 1.89 * 10^{-4} mol) and 84.5 mg (P3c: 3.15 * 10^{-4} mol) of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide dissolved in 10 mL THF. The solutions were stirred for 3 hours under nitrogen atmosphere at room temperature. Afterwards, 2 mL of ethylmethylamine (0.023 mol) were added into each flask. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The orange colored residues were suspended in 10 mL of water and each dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were twice dissolved in 10 mL of THF and precipitated in diethyl ether. Usually, after centrifugation and drying in a vacuum oven at 40 °C, between 0.54 g and 0.65 g of yellow-orange polymers were obtained. ^{1}H NMR (D_2O): δ /ppm: 7.76 (br s), 3.33 (br s), 2.84 (s), 1.63 (br s), 1.05 (s); FT-IR (ATR-mode): 3452 cm^{-1} (amide N-H-valence), 1623 cm^{-1} (C=O amide band I)

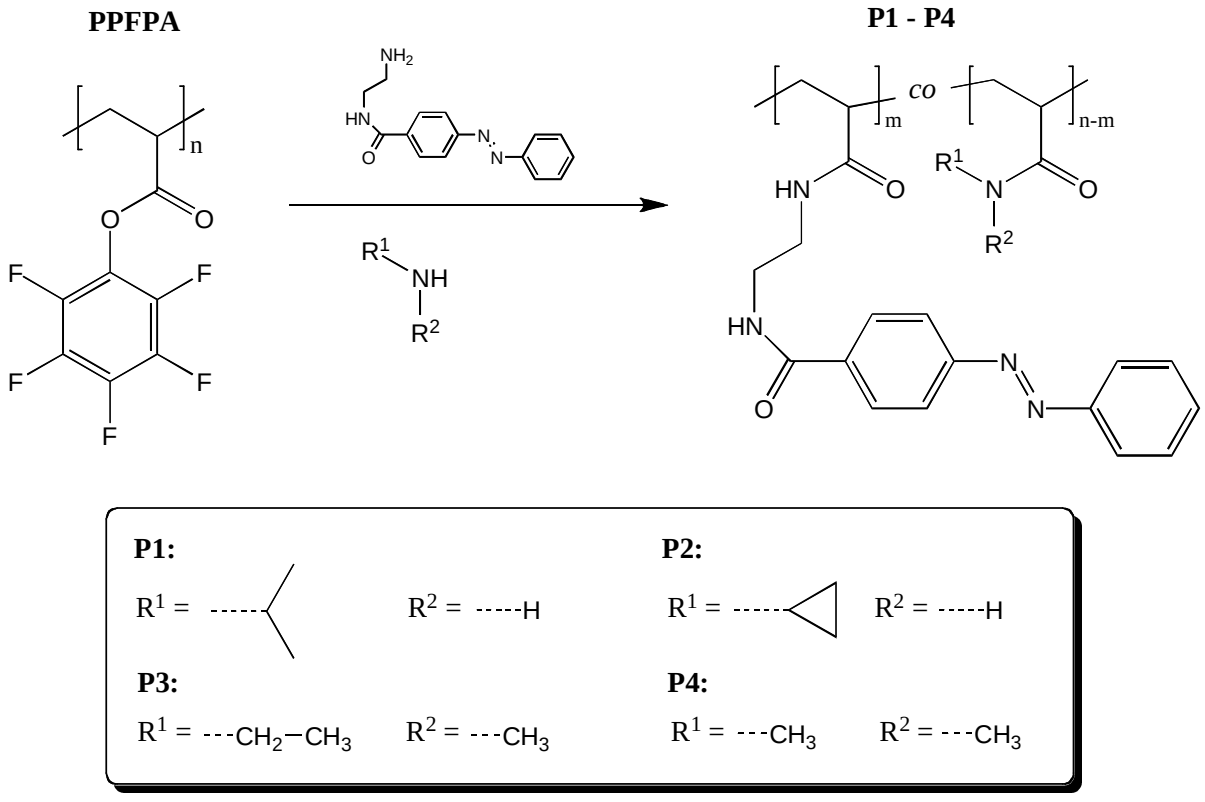
Synthesis of the temperature- and light sensitive copolymers P4. To three different solutions containing each 1.5 g of PPFPA and 2 mL triethylamine in 20 mL THF were added dropwise 84.5 mg (P4a: 3.15 * 10^{-4} mol), 135.1 mg (P4b: 5.04 * 10^{-4} mol) and 202.7 mg (P4c: 7.56 * 10^{-4} mol) of N-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide dissolved in 10 mL THF. The solutions were stirred for 3 hours under nitrogen atmosphere at room temperature. Afterwards, 8 mL of a 2.0 M solution of dimethylamine in THF (0.016 mol) were added into each flask and the solutions. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The orange colored residues were suspended in 10 mL of water and each dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were twice dissolved in 5 mL of a methanol/THF mixture (1:4) and precipitated in diethyl ether. Usually, after centrifugation and drying in a vacuum oven at 40° C, between 0.32 g and 0.52 g of yellow-orange polymers were obtained. ^{1}H NMR (D_2O): δ /ppm: 7.55 (br s), 2.90 (s), 2.62 (br s), 1.63 (br s), 1.34 (br s); FT-IR (ATR-mode): 3446 cm^{-1} (amide N-H-valence), 1622 cm^{-1} (C=O amide band I)

Results

To synthesize thermo- and light responsive polymers with varying amounts of azobenzene chromophores but maintaining the degree of polymerization, we made use of the polymer analogous reaction of poly(pentafluorophenylacrylate) (**PPFPA**) with amines. For that purpose, pentafluorophenylacrylate (PFPA) was synthesized as an activated ester monomer and polymerized under RAFT conditions yielding the corresponding reactive precursor polymer **PPFPA**. We conducted four polymerization experiments, which were carried out under the same conditions to obtain **PPFPA** polymers with the same molecular weight.

All resulting polymers had a narrow molecular weight distribution around 1.3, a molecular weight of around 25000 g/mol and were slightly pink colored due to their dithiobenzoate end-group. A defined degree of polymerization of 100 was chosen in order to exclude any influence of the molecular weight on the LCST.

In contrast to recent publication[26] the polymerization was performed for 20 hours to guarantee full conversion due to the large amount of 20 g of PFPA monomer. A broadening of the molecular weight distribution was not observed for longer polymerization times.



Scheme 1. *Synthetic scheme of four polyacrylamide series (P1 – P4) containing azobenzene moieties in varying amounts prepared by a polymer analogous reaction of the reactive precursor polymer poly(pentafluorophenylacrylate) with N-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide and isopropylamine, cyclopropylamine, diethylamine or ethylmethylamine, respectively.*

First, an azobenzene possessing an aliphatic amine-functionality necessary for a following polymer analogous reaction was synthesized in two steps. 4-(2-Phenyldiazenyl)benzoic acid was activated in the first step using commercial available pentafluorophenyl trifluoroacetate to yield the activated pentafluorophenyl 4-(2-phenyldiazenyl)benzoate in 63 % yield, which was then allowed to react in the next step with an excess amount of 1,2-diamino ethane. The final product was purified with several steps of washing with water to remove any excess of 1,2-diamino ethane and the pentafluorophenol salts. The resulting *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide was obtained in 82 % yield and had the required aliphatic amino-functionality to react with **PPFPA** activated precursor polymers in the next step.

The final product was purified with several steps of washing with water to remove any excess of 1,2-diamino ethane and the pentafluorophenol salts. The resulting *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide was obtained in 82 % yield and had the required aliphatic amino-functionality to react with **PPFPA** activated precursor polymers in the next step.

The polymer analogous reaction is shown in scheme 1. **PPFPA** polymers were dissolved in THF and allowed to react at room temperature with different amounts of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide in the presence of triethylamine (TEA). After 3 hours an excess amount of isopropylamine (**P1a-P1c**), cyclopropylamine (**P2a-P2d**), ethylmethylamine (**P3a-P3c**) or dimethylamine (**P4a-P4c**) was added and allowed to react for 12 hours to guarantee complete conversion, respectively. All resulting copolymers were dialyzed against diluted ammonia after the reaction to remove any formed pentafluorophenyl salts. The pure copolymers differed in the amount of incorporated azobenzene chromophore and the chemical compositions of the polyacrylamides are listed in table 1. Complete conversion of all reactions had been detected by IR-spectroscopy.

Table 1. *Composition and LCST values for the copolymer series P1-P4.*

Polymer	amount of azobenzene [mol%] *(calculated)*	amount of azobenzene [mol%] *(measured by UV/Vis)*	amount of azobenzene [mol%] *(measured by ^{1}H-NMR)*	LCST before irradiation [°C] *(measured by turbitomerty)*	LCST after irradiation [°C] *(measured by turbitomerty)*	Δ LCST [°C]
P1a	1.0	1.0	0.3	29.3	29.9	0.6
P1b	3.0	2.0	2.4	29.1	29.6	0.5
P1c	5.0	3.9	3.0	20.5	23.7	3.2
P2a	1.0	1.2	1.0	42.6	46.0	3.4
P2b	3.0	2.7	2.5	34.1	35.1	1.0
P2c	5.0	5.5	5.0	26.9	28.1	1.2
P2d	8.0	6.1	7.2	23.6	27.4	3.8
P3a	1.0	1.4	1.3	53.5	58.0	4.5
P3b	3.0	2.5	2.2	41.9	43.5	1.6
P3c	5.0	4.5	4.1	32.7	33.1	0.4
P4a	5.0	4.4	4.9	80.5	87.0	6.5
P4b	8.0	6.0	6.7	66.0	72.0	6.0
P4c	12.0	8.5	8.3	37.9	45.2	7.3

As an example, the IR-spectrum of polymer **P1c** is shown in figure 1. Full conversion of the polymer analogous reaction could be observed due to the complete vanishing of the activated ester polymer band at 1783 cm^{-1} and the appearance of the amides bands at 3302 cm^{-1}, 1644 cm^{-1} and 1537 cm^{-1}, respectively.

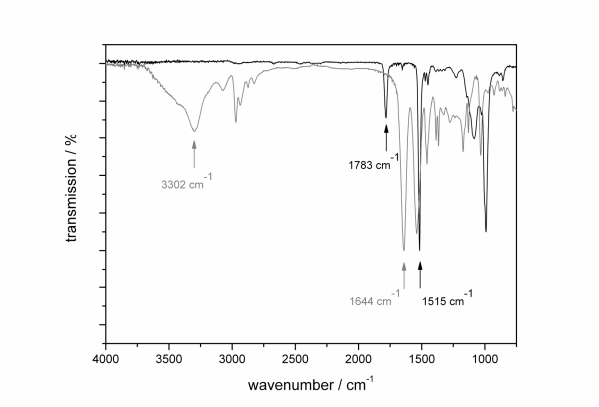


Figure 1. *IR spectra of PPFPA (black line) and the thermo- and light-responsive copolymer P1c (gray line).*

Additionally, ^{1}H NMR spectroscopy was used to characterize the copolymers. For example, the ^{1}H NMR of the copolymer **P1c** (see figure 2) showed the characteristic proton signals from the polyacrylamide backbone (in CD_2Cl_2: δ /ppm: 3.97, 1.11) and from the azobenzene protons (in CD_2Cl_2: δ /ppm: 8.07, 7.93, 7.52).

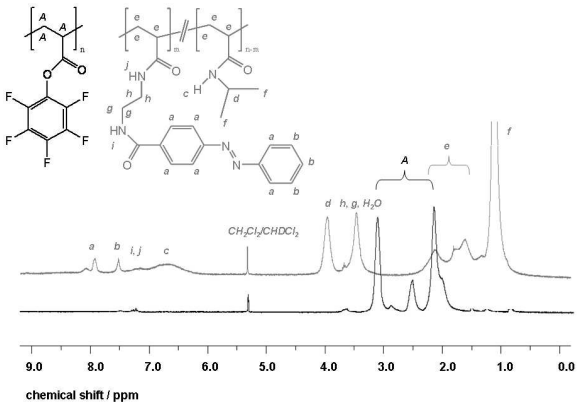


Figure 2. *^{1}H NMR of poly(pentafluorophenylacrylate) (black line) and the thermo- and light-responsive copolymer P1c (gray line) in CD_2Cl_2.*

Further, using ^{19}F NMR spectroscopy it was not possible to detect a fluorine signal anymore, indicating a complete conversion of all pentafluorophenyl ester groups. It also showed that the formed pentafluorophenol salts were totally removed through dialysis.

The GPC elugram in figure 3 shows the **PPFPA** and the copolymer **P1c**. The curve of **P1c** is shifted to higher elution volumes due to the lower molecular weight. Noteworthy, the molecular weight distribution did not change after the post modification.

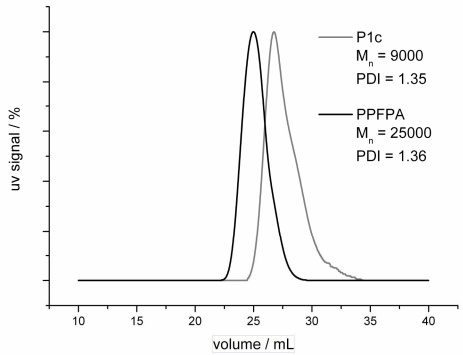


Figure 3. *GPC elugram of PPFPA (black line) and the copolymer P1c (gray line).*

The composition of the copolymers was additionally determined by UV/Vis spectroscopy assuming that the absorption coefficient of polymer bound azobenzene was identical to that of pentafluorophenyl 4-(2-phenyldiazenyl)benzoate. First, a calibration curve of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl) benzamide was calculated by measuring the absorption maximum at different concentrations in ethanol, and was then compared to the absorption maxima of the respective copolymers solutions of **P1-P4** in ethanol, respectively. The determined absorbances of the chromophoric copolymers were close to the theoretically calculated values and did not differ from the values measured by ^{1}H NMR spectroscopy. For the determination of the degree of substitution via ^{1}H NMR spectroscopy, the ratio of the integrals of the signals *d* (amide-signal) to *a* and *b* (azobenzene-signal) were used (see figure 2). All calculated and determined compositions of the copolymers are listed in table 1. In summary, we

have been able to prepare copolymers with varying amounts of azobenzene chromophore between 1-8.5 mol%.

Next, the light induced isomerization of the azobenzene group of the chromophoric copolymers was investigated in water. The UV/Vis spectrum of **P1c** in water, shown in figure 4, serves as an example for the kinetics of the light induced isomerization of the chromophores of all copolymers.

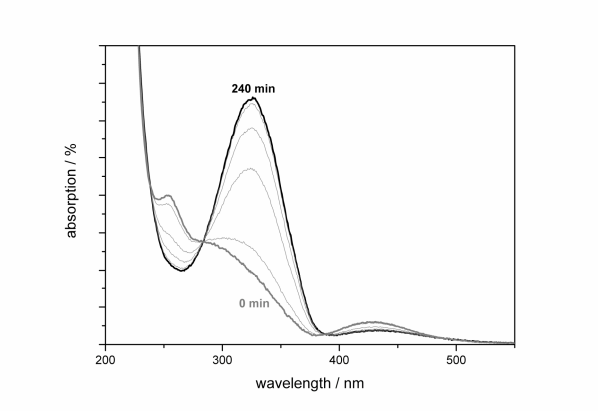


Figure 4. *Evolution of the UV/Vis spectrum of P1c in water (0.16 mg/mL) after irradiation at 365 nm for 1 h. The curves were measured at 0, 5, 30, 60, 120 and 240 min after irradiation.*

Prior irradiation all azobenzene groups were in the *trans*-configuration, which can be deduced from the characteristic absorption band at 326 nm. After irradiation of the sample with UV-light at 365 nm in a quartz cell for 1 hour, the absorption band at 326 nm decreased and the band for the exited *cis*-azobenzene at 256 nm appeared. The relaxation of the excited azobenzenes back into the *trans*-configuration occurred quickly ($\tau_{1/2}$ = 30 min) under visible light and reached equilibrium after 2 hours, with the majority of all chromophoric azobenzene side groups in the *trans*-configuration. In contrast to the light induced re-isomerization, the thermal relaxation in the dark was very slow at room temperature and had a half time of 11 days. In comparison to the re-isomerization observed for free azobenzene in chloroform, the values of the half times obtained for the polymer series **P1**-**P4** do not differ in more than 5 minutes.

The isomerization of azobenzene is always combined with a change in dipole moment of the molecular structure. Azobenzene groups are known to have a dipole moment of 0 debye in the *trans*-configuration, while azobenzene molecules in the *cis*-configuration have a dipole moment of 3 debye.[21] For the synthesized copolymers containing azobenzene groups, this means that the LCST can be shifted through irradiation with UV-light. Due to the increased dipole moment of *cis*-azobenzene, the respective copolymers showed a higher LCST for a *cis*-azobenzene containing polymer than for copolymers containing *trans*-azobenzene.[11-13] The LCSTs of the copolymer solutions were determined by turbidimetry, thus the optical transmittance of a light beam (λ = 632 nm) through the sample cell of the photospectrometer was monitored as a function of temperature. The concentration of all copolymer solutions was 20 mg/mL and the heating rate was 1 °C per minute. The cloud points were measured before and after irradiation with UV-light at 365 nm, respectively, and the LCST was defined as the temperature at which a transmission of 50 % was observed. The LCSTs of the aqueous solutions of the copolymers **P1**-**P4** exhibited a strong dependence upon the content of azobenzene moieties. In general, the LCST of all the copolymers decreased with increasing amount of azobenzene due to the hydrophobic character of *trans*-azobenzene. The LCSTs are listed in table 1. In all cases, higher LCST-values were observed after irradiation UV-light. This LCST shift upon irradiation can be explained by the isomerisation of the azobenzene groups accompanied with an increase in dipole moment and thus an increased local polarity present at the polymer backbone. Accordingly, within this temperature range an isothermal, light- induced precipitation of the

copolymers is possible.

As an example, the real-time photoswitching of the thermo- and light sensitive copolymers was demonstrated for the polymer **P4c** (see figure 5). A polymer solution of **P4c** (10 mg/mL) in a quartz cell was irradiated inside the spectrometer from above with UV-light of 365 nm by using an optical fiber at a constant temperature higher than the LCST (T = 40 °C). The optical transmittance of the light beam (λ = 632 nm) was recorded versus the irradiation time.

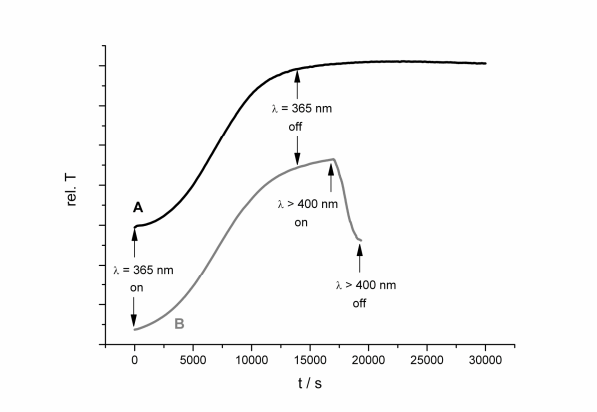


Figure 5. *Real-time photoswitching experiment of the copolymer P4c at 40 ° C. A: The black curve shows the change of the transmittance during irradiation with UV-light (from 0 to 14000 s) and following thermal relaxation in the dark (from 21000 to 30000 s). B: The grey curve shows the change of the transmittance during irradiation with UV-light (from 0 to 14000 s) and following light induced reisomerization (λ > 400 nm, from 16700 to 19000s).*

During the irradiation time of 4 h with UV-light, the transmittance of the solution at 40 °C increased from 8 % up to 92 % intensity due to higher solubility of the polymer with azobenzene groups in the *cis*-configuration. This shows the light responsive phase transition behavior of the polymer. With the azobenzene groups in the *cis*-configuration, re-isomerization is possible through thermal relaxation in the dark, as can be seen by the slow decrease of the transmittance intensity after the irradiation with UV-light has been turned off (see figure 5, curve A). The process occurred very slow and had a lifetime of several days. In contrast to the thermal relaxation, the light induced re-isomerization occurred very fast. The optical transmittance of the solution decreased quickly while irradiating the sample cell with visible light (λ > 400 nm) using an optical filter (see figure 5, curve B). Consequently, a halftime of 40 min for the light induced reisomerization could be calculated. This value fits to the results from the UV/Vis kinetic experiment mentioned above. Therefore the real-time light controlled solubility change of polymer **P4c** could be demonstrated.

Discussion

For the copolymer series **P1**, we observed the highest LCST shift (3.2 °C) for an amount of 3.9 mol% azobenzene (**P1c**) compared to the LCST shift of 0.6 °C (1.0 mol% azobenzene) for **P1a** and 0.5 °C (2.0 mol% azobenzene) for **P1b**. In contrast, the LCST for *trans*-azobenzene polymers did not differ in more than 0.2 °C for a content between 1.0 mol% (**P1a**) to 2.0 mol% (**P1b**) of azobenzene. Only with higher a content of *trans*-azobenzene of 3.9 mol% (**P1 c**), the LCST was decreased tremendously. This phenomenon of a sudden change of the LCST at a critical azobenzene content of 3 mol% was already described and reported by Irie and Kungwatchakun.[17] By incorporation of azobenzene chromophores into the polymer, the hydrophobic interaction is enforced, and the polymer becomes less soluble. This resulted in a decrease of the LCST from **P1a** to **P1c**. Accordingly, at higher contents of azobenzene the light-induced isomerization into the less hydrophobic *cis*-azobenzenes increased the phase separation temperature up to 3.2 °C.

For the copolymer series **P2** no tendency for the LCST shifts in correlation to the amount of azobenzene chromophores was found. The LCSTs of the series of *trans*-azobenzene copolymers **P2** decreased almost linear with increasing amount of

chromophores. However, the polymers with the lowest (1.2 mol %) and the highest amount (6.1 mol %) of incorporated azobenzene showed the highest differences in LCST before and after irradiation.

The copolymers **P3** also showed a linear decrease of the LCSTs with increasing amount of hydrophobic *trans*-azobenzene. But in the case of **P3a** with an incorporated azobenzene amount of 1.4 mol%, the highest LCST shift of 4.5 °C before and after irradiation was observed. In contrast to the copolymer series **P1** the shifts of the LCST decreased with increasing amounts of chromophore.

The copolymer series **P4**, based on poly(*N*,*N*-dimethylacrylamide), showed the highest differences in the LCST between the irradiated and non-irradiated samples. For copolymer **P4c** with an amount of 8.5 mol % of incorporated azobenzene, the highest difference in the LCST of 7.3 °C was measured. Further, it has to be mentioned that the LCST shifts of all copolymers **P4a-P4c** seemed to be almost independent of the amount of incorporated azobenzene, as the shift of LCST did not differ in more than 1.3 °C for varying amounts of incorporated azobenzene between 4.0 and 8.5 mol%. Even though, similar to the copolymer series **P2** and **P3**, the LCSTs showed a linear decrease with increasing amount of incorporated chromophore. In general, the shift in the LCST seemed to be dependent from the chemical structure of the different copolymers **P1-P4** and from the amount of incorporated azobenzene.

In comparison to the thermo- and photoresponsive poly(acrylamides) that had been prepared by free radical polymerization, our measured values correspond very well with those measured by Kroeger et al.[11] and thus are a beautiful example of the synthetic versatility of the polymer analogous reaction of **PPFPA** with functional amines.

Conclusion

In summary, we have presented the synthesis of copolymers that exhibit a thermo- and light responsive behavior in aqueous solution. Within the investigated polymers, the degree of polymerization had been kept constant, which could be achieved by RAFT polymerization of pentafluorophenylacrylate yielding reactive precursor polymers with defined molecular weight, i.e. degree of polymerization and a narrow molecular weight distribution. Four series of polyacrylamides containing azobenzene moieties in varying amounts have been prepared by a polymer analogous reaction of the reactive precursor polymer poly(pentafluorophenylacrylate) with *N*-(2-aminoethyl)-4-(2-phenyl-diazenyl) benzamide and isopropylamine, cyclopropylamine, diethylamine or ethylmethylamine, respectively. The obtained copolymer series **P1-P4** exhibited a LCST in aqueous solution that depended strongly on the amount of incorporated azobenzene. Furthermore, the reversible isomerization of the azobenzene groups in the copolymers, which was induced by irradiation with UV-light, had an influence on the LCST. Higher LCST values were measured after irradiation and thus, in the temperature region between the LCST of the non-irradiated and the irradiated solution, a light controlled reversible solubility change was observed. The light controlled solubility change could be demonstrated in a real-time experiment. In general, it can be concluded that the copolymers, which have been prepared by polymer analogous reaction with the azobenzene derivate *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide showed a similar response behavior than the previously reported copolymerization of e. g. *N*-isopropylacrylamide with *N*-(4-phenyl-azophenyl)acrylamide, demonstrating that the presented polymer analogous synthesis of light- and temperature responsive polymers provides a much more versatile synthetic route.

Acknowledgements

University of Mainz is gratefully acknowledged for financial support. S. Bonenberger is acknowledged for support in the experimental work.

References

(1) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163-249.

(2) Plate, N. A.; Lebedeva, T. L.; Valuev L. I. *Polymer Journal* **1999**, *31*, 21-27.

(3) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2001**, *34*, 1391-1399.

(4) Idziak, I.; Avoce, D.; Lessard, D. *Macromolecules* **1999**, *32*, 1260-1263.

(5) Okano, T.; Bae, Y. H.; Kim, S. W. *J. Controlled Release* **1990**, *11*, 255-265.

(6) Chen, J. P.; Yang, H. J.; Hoffman, A. S. *Biomaterials* **1990**, *11*, 625-630.

(7) Li, D.; He, Q.; Yang, Y.; Moehwald, H.; Li, J. *Macromolecules* **2008**, 41, 7254-7256.

(8) Magnusson, J. P.; Khan, A.; Pasparakis, G.; Saeed, A.; Wang, W.; Alexander, C. *J. Am. Chem. Soc.* **2008**, *130*, 10852-10853.

(9) Wang, G.; Tong, X.; Zhao, Y. *Macromolecules* **2004**, *37*, 8911-8917.

(10) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(11) Kroeger, R.; Menzel, H.; Hallensleben, M. L. *Macromol. Chem. Phys.* **1994**, *195*, 2291-2298.

(12) Akiyama, H.; Tamaoki, N. *J. Polym. Sci. A. Polym. Chem.* **2004**, *42*, 5200-5214.

(13) Kungwatchakun, D.; Irie, M. *Makromol. Chem. Rapid Commun.* **1988**, *9*, 243-246.

(14) Akiyama, H.; Tamaoki, N. *Macromolecules* **2007**, *40*, 5129-5132.

(15) Chunhua, L.; Fang, Z.; Zhaohui, Z.; Yuxing, P. *J. Appl. Polym. Sci.* **2007**, *107*, 2118-2125.

(16) Shimoboji, T.; Larenas, E.; Fowler, T.; Hoffman, A. S.; Stayton, P. S. *PNAS* **2002**, *99*, 16592-16596.

(17) Irie, M.; Kungwatchakun, D. *Proc. Japan Acad. Ser. B* **1992**, *68*, 127-132.

(18) Luo, C.; Zuo, F.; Ding, X.; Zheng, Z.; Cheng, X.; Peng, Y. *J. Appl. Polym. Sci.* **2008**, *107*, 2118-2125.

(19) Hartley, G. S. *Nature* **1937**, *140*, 281.

(20) Yager, K. G.; Barrett, C. J. *Journal of Photochemistry and Photobiology A: Chemistry* **2006**, *182*, 250-261.

(21) Hartley, G. S.; LeFevre R. J. W. *J. Chem. Soc.* **1939**, 531-534.

(22) Eberhardt, M.; Mruk, R.; Zentel, R.; Theato P. *Eur. Polym. J.* **2005**, *41*, 1569-1575.

(23) Metz, N.; Theato, P. *Eur. Polym. J.* **2007**, *43*, 1202-1209.

(24) Nilles, K.; Theato, P. *Eur. Polym. J.* **2007**, *43*, 2901-2912.

(25) Theato, P. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 6677-6687.

(26) Eberhardt, M. Theato, P. *Macromol. Rapid Commun.* **2005**, *26*, 1488-1493.

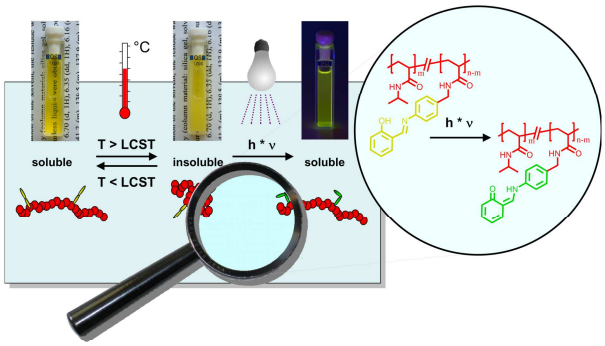
(27) Chong, Y. K.; Krstina, J.; Le, T. P.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2003**, *36*, 2256-2272.

Temperature- and Light-Responsive Polyacrylamides Prepared by a Double Polymer Analogous Reaction of Activated Ester Polymers

*Florian D. Jochum, Patrick Theato**

ABSTRACT

Two different series of polyacrylamides containing different amounts of salicylideneaniline moieties have been synthesized via a double polymer analogous reaction of poly(pentafluorophenyl acrylate) (PPFPA). All copolymers were designed to exhibit a lower critical solution temperature (LCST) in aqueous solution, which was dependent on (i) the amount of incorporated chromophoric salicylideneaniline groups and (ii) the isomerization state of the respective salicylideneaniline group. Higher LCST values were measured for UV-irradiated solutions of the copolymers in comparison to the non-irradiated copolymer solutions. A maximum difference in the LCST of up to 13°C was found for poly(*N*-cyclopropylacrylamide) copolymer containing 15.0 mol% of salicylideneaniline groups. Within this temperature range, a reversible solubility change of the copolymer could be induced by irradiation with light.



Introduction

Stimuli-responsive polymers -- so called smart polymers -- have attracted great interest in academic and applied science recently. Most commonly, approaches take advantage of thermally induced, reversible phase transitions. In this context, polymers based on polyacrylamides found great interest as thermo-responsive polymers in aqueous solution. Many polyacrylamides, e.g. poly(*N*-isopropylacrylamide) (PNIPAM)[1,2] or poly(*N*-cyclopropylacrylamide)[3], feature a sharp transition behaviour in water in response to changes of the temperature. Accordingly, such polymers featuring a lower critical solution temperature (LCST) in water are expected to find application in many scientific areas. As an example drug delivery[4-9] or immobilization of enzymes and cells[10-12] can be mentioned.

Besides polymers that are responsive to a single stimulus, recently research on polymers that show a responsive behavior to multiple stimuli has been intensified[13,14]. Other stimuli besides temperature can for example be pH[15], ionic strength[16] or light[17,18]. Very appealing and important for applications are polymers that are responsive to light and temperature and accordingly, there have been several reports on temperature-responsive copolymers that contain a light-responsive moiety, for example azobenzene groups[19-27].

In the following, the synthesis of temperature- and light-responsive polyacrylamide copolymers featuring salicylideneaniline as a photochromic group is reported. Salicylideneaniline is known to isomerise upon irradiation with UV-light from the *enol*-form into the *keto*-form, which results in a change of the dipole moment[28-34]. Two different series of thermo- and light-responsive polyacrylamide derivates that take advantage of salicylideneaniline as the corresponding photochromic group will be synthesized and characterized. The synthesis extends the thorough investigated polymer analogous reaction of activated ester polymers as tools for functional polymers[35-39] to a double polymer analogous reaction of poly(pentafluorophenyl acrylate) (PPFPA).

Experimental Section

Materials. All chemicals and solvents were commercially available and used as received unless otherwise stated. Tetrahydrofurane (THF), 1,4-dioxane and diethyl ether were previous distilled over sodium. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at -7°C. As dialysis membranes Spectra/Por 3 (MWCO 3500) were used. Benzyl dithiobenzoate was synthesized as described in the literature[40].

Instrumentation. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz FT-NMR spectrometer in deuterated solvents. ^{19}F NMR spectra were recorded on a Bruker 400 MHz FT-NMR spectrometer. Chemical shifts (δ) were given in ppm relative to TMS. Gel permeation chromatography (GPC) was used to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards. GPC measurements were performed in THF as solvent and with the following parts: pump PU 1580, auto sampler AS 1555, UV-detector UV 1575, RI-detector RI 1530 from Jasco, and miniDAWN Tristar light scattering detector from Wyatt. Columns were used from MZ-Analysentechnik: MZ-Gel SDplus 102 Å, MZ-Gel SDplus 104 Å and MZ-Gel SDplus 106 Å. The elution diagrams were analyzed using the ASTRA 4.73.04 software from Wyatt Technology. Calibration was done using polystyrene standards. The flow rate was 1 mL/min at a temperature of 25°C. UV/Vis spectra were recorded on a Jasco V-630 photospectrometer. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer using an ATR unit. FD-masses were

measured on a MAT 95 Finnigan mass spectrometer. Cloud points were determined in Millipore water at a concentration of 40 mg/mL and were observed by optical transmittance of a light beam (λ = 632 nm) through a 1 cm sample quartz cell. The measurements were performed in a Jasco V-630 photospectrometer with a Jasco ETC-717 peltier element. The intensities of the transmitted light were recorded versus the temperature of the sample cell. The heating rate was 1°C per minute. Irradiation experiments of the polymer solutions were performed in the sample cell of the photospectrometer using an Oriel Instruments 500 W mercury lamp with a 365nm filter and an optical fiber (see scheme 2).

Pentafluorophenylacrylate (PFPA). 80 g (0.43 mol) pentafluorophenol and 52.5 g (0.52 mol) triethylamine (TEA) were dissolved in 500 mL dry diethyl ether and 47.2 g (0.52 mol) acryloyl chloride was added dropwise through a funnel under cooling with an ice bath. After stirring additional 2 hours at room temperature, the precipitated salt was removed by filtration. After evaporation of the solvent, the residue was filtered again and purified with column chromatography (column material: silica gel, solvent: petroleum ether). 75 g (0.32 mol – 74 %) of a colourless liquid were obtained. The pure PFPA was stored at -7°C. ^{1}H NMR ($CDCl_3$): δ /ppm: 6.70 (d, 1H), 6.35 (dd, 1H), 6.16 (d, 1H); ^{13}C NMR ($CDCl_3$): δ /ppm: 161.5 (s), 142.8 (m), 141.2 (m), 139.5 (m), 137.9 (m), 136.2 (m), 134.9 (s), 125.1 (s); ^{19}F NMR (CDCl3): δ /ppm: -162.77 (d, 2F), -158.39 (t, 1F), -153.02 (d, 2F); FT-IR (ATR-mode): 1772 cm^{-1} (C=O reactive ester band), 1516 cm^{-1} (C=C aromatic band)

Poly(pentafluorophenylacrylate) (PPFPA). In a typical RAFT polymerization a mixture of 20 g (0.084 mol) PFPA, 51.5 mg (2.11 * 10^{-4} mol) benzyl dithiobenzoate and 4.3 mg (2.62 * 10^{-5} mol) AIBN were placed into a Schlenk-flask. After addition of 50 mL dry 1,4-dioxane, four freeze-pump-thaw cycles were performed to degas the solution. The flask was filled with argon, immersed in a preheated oil bath of 80°C and stirred overnight. After cooling down to room temperature, the polymer was isolated by precipitation into hexane. The crude polymer was dissolved in THF, precipitated again twice into hexane, centrifuged and finally dried in a vacuum oven at 40°C. Yield 13 g (65 %) of a pink powder. ^{1}H NMR ($CDCl_3$): δ /ppm: 3.10 (br s), 2.51 (br s), 2.13 (br s); ^{13}C NMR ($CDCl_3$): δ /ppm: 169.28 (s), 142.24 (m), 138.93 (m), 135.77 (m), 40.11 (br s), 34.27 (br s); ^{19}F NMR ($CDCl_3$): δ /ppm: -162.28 (br s), -157.15 (br s), -153.56 (br s); FT-IR (ATR-mode): 1783 cm^{-1} (C=O reactive ester band), 1515 cm^{-1} (C=C aromatic band), 1090 cm^{-1} (C-O ester band)

Synthesis of the copolymers P1. To two different solutions containing each 1.5 g of PPFPA and 2 mL TEA in 20 mL THF were added dropwise 7.6 mg (P1a: 6.30 * 10^{-5} mol), and 30.8 mg (P1b: 2.52 * 10^{-4} mol) of 4-aminobenzylamine dissolved in 5 mL THF. The solutions were stirred for 2 hours under nitrogen atmosphere at room temperature. Afterwards, 2 mL of isopropylamine (0.023 mol) were added into each flask. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The colorless residues were suspended in 10 mL of water and dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were three times dissolved in 5 mL of THF and precipitated into hexane. Usually, after centrifugation and drying in a vacuum oven at 40°C, 0.35 g and 0.39 g of colorless polymers were obtained. ^{1}H NMR (CD_2Cl_2): δ /ppm: 7.06 (s), 6.64 (s), 6.27 (br s), 4.23 (br s), 3.98 (s), 2.10 (br s), 1.60 (br s), 1.12 (s); FT-IR (ATR-mode): 3306 cm^{-1} (amide N-H-valence), 2971 cm^{-1} (C-H valence

band), 1640 cm^{-1} (C=O amide band I), 1533 cm^{-1} (C=O amide band II), 838 cm^{-1} (aromatic 1,4-disubstitution)

Synthesis of the copolymers P2. To four different solutions containing each 1.5 g of PPFPA and 2 mL TEA in 20 mL THF were added dropwise 15.4 mg (P2a: 1.26 * 10^{-4} mol), 38.4 mg (P2b: 3.15 * 10^{-4} mol), 76.9 mg (P2c: 6.30 * 10^{-4} mol) and 115.3 mg (P2d: 9.45 * 10^{-4} mol) of 4-aminobenzylamine dissolved in 5 mL THF. The solutions were stirred for 2 hours under nitrogen atmosphere at room temperature. Afterwards, 2 mL of cyclopropylamine (0.029 mol) were added into each flask. After additional 18 hours of stirring, the solvent of each sample was removed by evaporation in vacuum. The colorless residues were suspended in 10 mL of water and dialyzed against diluted ammonia over night. The dialyzed solutions were evaporated and the residues were three times dissolved in 5 mL of methanol and precipitated into diethyl ether. Usually, after centrifugation and drying in a vacuum oven at 40°C, between 0.33 g and 0.54 g of colorless polymers were obtained. ^{1}H NMR (MeOD): δ /ppm: 8.03 (br s), 7.09 (s), 6.73 (s), 4.25 (br s), 2.72 (s), 2.07 (br s), 1.59 (br s), 0.66 (d); FT-IR (ATR-mode): 3293 cm^{-1} (amide N-H-valence), 2971 cm^{-1} (C-H valence band), 1646 cm^{-1} (C=O amide band I), 1517 cm^{-1} (C=O amide band II), 826 cm^{-1} (aromatic 1,4-disubstitution)

Synthesis of the temperature- and light sensitive copolymers P3. To two different solutions containing each 300 mg of P1a and P1b in 10 mL ethanol were added dropwise 2 mL (0.019 mol) of salicylaldehyde. The solutions were stirred for 2 hours under nitrogen atmosphere at room temperature. Afterwards, the solvent of each sample was removed by evaporation in vacuum. The yellow residues were three times dissolved in 5 mL of THF and precipitated into hexane. Usually, after centrifugation and drying in a vacuum oven at 40°C, 0.18 g and 0.27 g of yellow polymers were obtained. ^{1}H NMR (CD_2Cl_2): δ /ppm: 13.20 (s), 8.67 (s), 7.38 (br s), 6.95 (s), 6.37 (br s), 4.41 (br s), 4.00 (s), 3.35 (br s), 2.08 (br s), 1.65 (br s), 1.12 (s); FT-IR (ATR-mode): 3302 cm^{-1} (amide N-H-valence), 2981 cm^{-1} (C-H valence band), 1644 cm^{-1} (C=O amide band I), 1540 cm^{-1} (C=O amide band II), 747 cm^{-1} (aromatic 1,2-disubstitution)

Synthesis of the temperature- and light sensitive copolymers P4. To four different solutions containing each 300 mg of P2a, P2b, P2c and P2c in 10 mL ethanol were added dropwise 2 mL (0.019 mol) of salicylaldehyde. The solutions were stirred for 2 hours under nitrogen atmosphere at room temperature. Afterwards, the solvent of each sample was removed by evaporation in vacuum. The yellow residues were three times dissolved in 5 mL of methanol and precipitated into diethyl ether. Usually, after centrifugation and drying in a vacuum oven at 40°C, between 0.21 g and 0.26 g of yellow polymers were obtained. ^{1}H NMR (MEOD): δ /ppm: 8.78 (br s), 7.38 (br s), 6.95 (s), 4.39 (br s), 2.70 (s), 2.07 (br s), 1.60 (br s), 0.67 (d); FT-IR (ATR-mode): 3287 cm^{-1} (amide N-H-valence), 2968 cm^{-1} (C-H valence band), 1645 cm^{-1} (C=O amide band I), 1527 cm^{-1} (C=O amide band II), 756 cm^{-1} (aromatic 1,2-disubstitution)

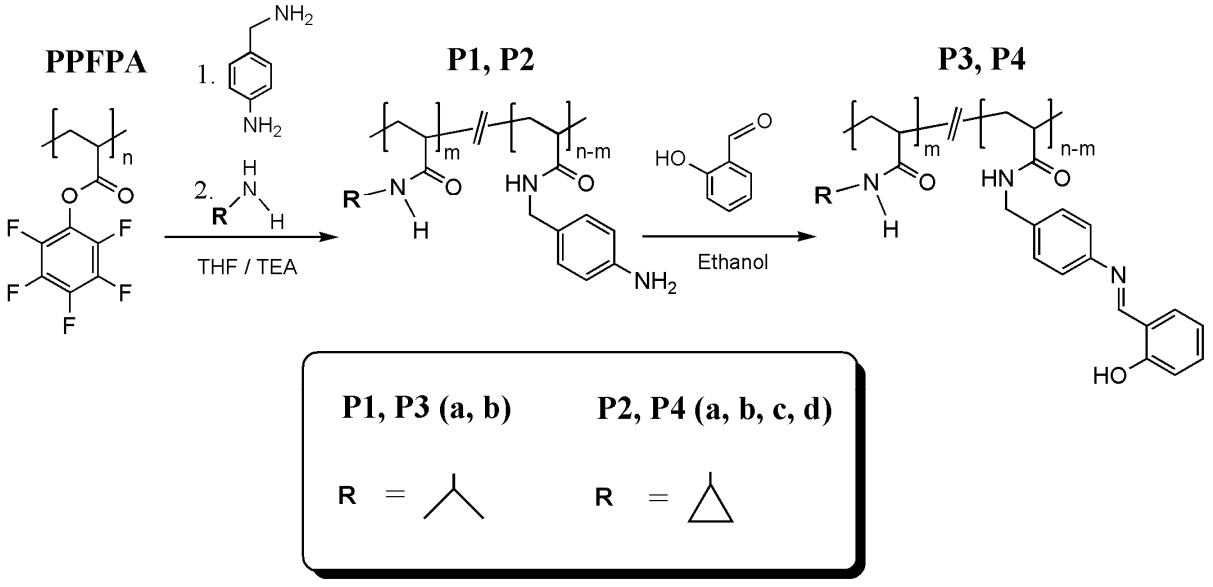
Synthesis of 4-(2-hydroxybenzylidene-amino)benzoic acid. 5.42 g (0.0396 mol) of 4-aminobenzoic acid were dissolved in 120 mL ethanol and 4.83 g (0.0396 mol) of salicylaldehyde in 80 mL ethanol were added rapidly under magnetically stirring. After several minutes, the final yellow product started to precipitate. The mixture was totally stirred 2 hours at room temperature and the precipitated solid was isolated by filtration through a glass frit. The product was washed with ethanol and dried in a vacuum oven at 40°C. 8.5 g (0.0353 mol – 89 %) of yellow 4-(2-hydroxybenzylideneamino)benzoic acid were

obtained. ^{1}H NMR (DMSO): δ /ppm: 12.79 (br s, 1H), 8.98 (s, 1H), 8.01 (d, 2H), 7.68 (d, 1H), 7.47 (d, 2H), 7.42 (d, 1H), 6.99 (m, 2H); FT-IR (ATR-mode): 1679 cm^{-1} (C=O aryl acid band), 1600 cm^{-1} (C=C aromatic valence), 1569 cm^{-1} (C=C aromatic valence), 1287 cm^{-1} (C-O band), 860 cm^{-1} (aromatic 1,4-disubstitution), 751 cm^{-1} (aromatic 1,2-disubstitution); MS (FD) m/z (%): 241.8 (12.97), 240.8 (100.00)

Results and Discussion

First, poly(pentafluorophenylacrylate) (**PPFPA**) was synthesized by a reversible addition-fragmentation chain transfer (RAFT) polymerisation utilizing benzyl dithiobenzoate as a chain transfer agent in 1,4-dioxane. The resulting polymer was obtained by precipitation and had molecular weight M_n = 24.900 g/mol with a narrow molecular weight distribution (M_w/M_n = 1.36).

PPFPA was then subjected to a first polymer analogous reaction with different amounts of 4-aminobenzylamine in tetrahydrofuran at room temperature (see scheme 1). After two hours of reaction, the remaining pentafluorophenyl ester groups were converted with an excess amount of isopropylamine or cyclopropylamine, respectively, yielding two series of temperature-responsive polyacrylamide derivates. 4-aminobenzylamine will react selectively with its aliphatic amino-group, because the nucleophilicity of the aromatic amino-group is not sufficient to attack the activated ester polymer, as we have reported recently[35]. The two resulting copolymer series **P1** and **P2** were purified by dialysis against dilute ammonia as well as precipitation to remove any pentafluorophenol salts. All polymers were analyzed by FT-IR-spectroscopy, GPC measurement and NMR-spectroscopy. As an example, the IR-spectrum of copolymer **P1b** is shown in figure 1.



Scheme 1. *Synthetic scheme of two polyacrylamide series (P3 and P4) containing salicylideneaniline moieties in varying amounts prepared by a double polymer analogous reaction of the reactive precursor polymer poly(pentafluorophenylacrylate) (PPFPA).*

The quantitative conversion of the polymer analogous reaction was confirmed by the complete vanishing of the activated ester polymer band at 1783 cm^{-1} of the **PPFPA** and the appearance of two amide bands at 1640 cm^{-1} and 1533 cm^{-1} of the copolymer **P1b**, respectively.

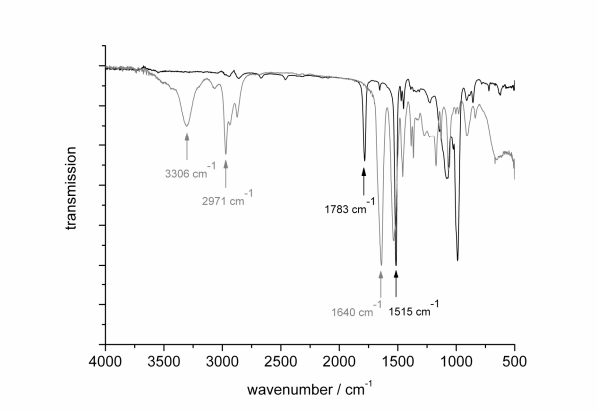


Figure 1. *FT-IR spectra of PPFPA and the copolymer P1b (gray line).*

Additionally, the appearance of the NH-group valence vibrations in the infrared spectrum appeared at 3306 cm^{-1}. In figure 2, the GPC elugram of **PPFPA** and the copolymer **P1b** are shown. After the first polymer analogous reaction, the GPC trace of **P1b** (M_n(P1b) = 9400 g/mol) is shifted to lower molecular weights in comparison to the GPC trace of **PPFPA** (M_n(PPFPA) = 24900 g/mol) due to the decrease of molecular weight per repeating unit.

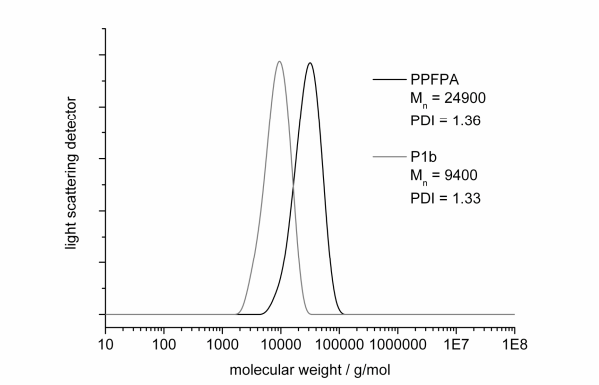


Figure 2. *GPC elugram of poly(pentafluorophenyl acrylate) (black line) and the copolymer P1b (gray line).*

However, the obtained value for the molecular weight of the polymer **P1b** (M_n(P1b) = 9400 g/mol) is slightly lower than the theoretical value (M_n(theo.) = 11800 g/mol) calculated from the ^{1}H-NMR data of the copolymer composition taking the molecular weight of **PPFPA** into account. A suitable explanation for this divergence might result from the calibration of the GPC with respect to polystyrene standards. Nevertheless a successful conversion could be demonstrated from the GPC shift of the polymer **P1b**. Noteworthy, the molecular weight distribution did not change after the post-modification. The conversion was also followed by ^{1}H NMR spectroscopy and as an example, the ^{1}H NMR of copolymer **P1b** is shown in figure 3.

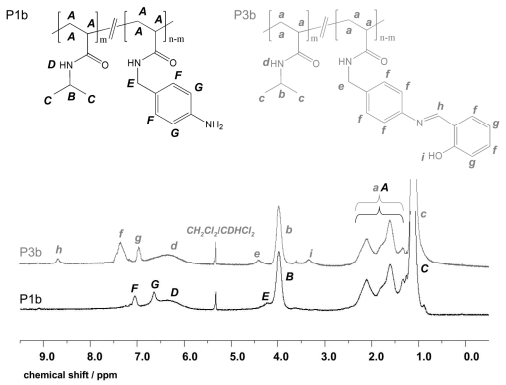


Figure 3. *^{1}H NMR of the copolymer P1b (black line) and the thermo- and light-responsive copolymer P3b (gray line) in CD_2Cl_2.*

All characteristic proton signals from the polyacrylamide side group (in CD_2Cl_2: δ /ppm: 6.27, 3.98, 1.12) and from the aromatic aniline protons (in CD_2Cl_2: δ /ppm: 7.06, 6.64) can be identified. Further, ^{19}F NMR spectroscopy was not able to detect a fluorine signal anymore, indicating a complete conversion of all pentafluorophenyl ester groups. It also showed that all pentafluorophenol salts were removed during the purification by dialysis.

In the second polymer analogous reaction (see scheme 1), the aniline functional group of the copolymer series **P1** and **P2** was converted selectively with salicylaldehyde in ethanol solution

at room temperature to yield the photoswitchable salicylideneaniline group. The solution turned immediately yellow upon addition of salicylaldehyde, indicating the successful formation of salicylideneaniline chromophores. The resulting copolymer series **P3** and **P4** were isolated by precipitation into hexane or diethylether and dried in vacuum. A successful conversion of this second polymer analogous reaction has been confirmed by ^{1}H NMR spectroscopy and UV/Vis spectroscopy. The ^{1}H NMR spectrum of **P3b** is shown in figure 3 and the characteristic proton signals of the formed salicylideneaniline Schiff's base can be assigned (in CD_2Cl_2: δ /ppm: 8.67, 7.38, 6.95). Additionally, the signals of the aromatic aniline protons (in CD_2Cl_2: δ /ppm: 7.06, 6.64) of **P1b** totally disappeared, indicating a complete conversion.

As an example, the UV/Vis spectrum of the copolymer P3b in ethanol was measured (see supporting information, figure S1) and compared to the UV/Vis spectrum of 4-(2-hydroxy-benzylideneamino)benzoic acid. Both spectra showed the characteristic absorption peaks at 270 nm, 302 nm, 320 nm and 340 nm of the respective salicylideneaniline, indicating the formation of the chromophore. Table 1 summarizes the list of synthesized copolymers with varying contents of salicylideneaniline ranging from 1 mol% to 15 mol%.

The amount of incorporated chromophore has been determined by ^{1}H NMR spectroscopy. The integrals of the aromatic signals (7.38 ppm, 6.95 ppm) of the salicylideneaniline group and the single signal of the polyacrylamide protons (**P3** series: 4.00 ppm, **P4** series: 2.70 ppm) were used for the calculation.

The composition of the copolymers was additionally determined by UV/Vis spectroscopy assuming that the absorption coefficient of polymer bound salicylideneaniline was identical to that of 4-(2-hydroxybenzylideneamino)benzoic acid at 277 nm. A calibration curve of 4-(2-hydroxybenzylideneamino)benzoic acid was calculated by measuring the absorption maximum at different concentrations in ethanol. Comparison to the absorption maxima at 277 nm of the copolymers solutions **P3** and **P4** in ethanol yielded the incorporation ratio of chromophore.

Table 1. *Composition and LCST values for the copolymer series P3 and P4.*

Polymer	amount of salicylidene-aniline [mol%] *(calculated)*	amount of salicylidene-aniline [mol%] *(measured by UV/Vis)*	amount of salicylidene-aniline [mol%] *(measured by ^{1}H-NMR)*	LCST before irradiation [°C] *(measured by turbidimetry)*	LCST during irradiation [°C] *(measured by turbidimetry)*	Δ LCST [°C]
P3a	1.0	0.4	1.0	29.7	30.6	0.9
P3b	4.0	3.8	4.0	26.3	30.6	4.3
P4a	2.0	2.4	2.0	41.8	46.4	4.6
P4b	5.0	3.3	4.0	36.5	38.5	2.0
P4c	10.0	9.6	9.0	33.9	36.0	2.1
P4d	15.0	13.7	15.0	25.1	38.1	13.0

The determined chromophore incorporation ratios of the copolymers were close to the theoretically calculated values and did not significantly differ from the values measured by ^{1}H NMR spectroscopy (see table 1), demonstrating the efficiency of the two polymer analogous reactions.

The LCSTs of the copolymer series **P3** and **P4** were determined by turbidimetry, thus, the optical transmittance of a light beam (λ = 632 nm) through the sample cell of the photospectrometer was monitored as a function of temperature. The concentration of all copolymer solutions was 40 mg/mL in Millipore water and the heating rate was 1 °C per minute. The cloud points were measured before and during irradiation with UV-light (λ = 365 nm) using an optical fiber (see scheme S2, supporting information). The LCST was defined as the temperature at which a transmission of 50 % was observed. The LCSTs of the aqueous solutions of the copolymer series **P3** and **P4** exhibited a strong dependence upon the content of incorporated salicylideneaniline. In general, the LCST of all copolymers decreased almost linear with increasing amount of salicylideneaniline, due to the hydrophobic character of the chromophore. The LCSTs are listed in table 1. In all cases, higher LCST-values were observed during irradiation with UV-light. Therefore, the LCST shift upon irradiation can be explained by the isomerisation of the salicylideneaniline groups from the *enol*- to the *keto*-form, accompanied with an increase in dipole moment and thus an increased local polarity present at the polymer backbone, which results in the increase of LCST. Accordingly, within the temperature range of the LCSTs before and after irradiation, an isothermal, light-induced precipitation of the copolymers was possible. By turning off the UV-light, the respective LCSTs values remained higher than before irradiation which might be explained by an intramolecular stabilization of the exited *keto*-form in high polar media such as water. After evaporation of the samples solutions and re-dissolving in water, the values for the LCST as before irradiation was performed were measured again. Any decomposition of the salcylideneaniline moieties during the irradiation experiment could be excluded by a careful investigation via ^{1}H NMR-spectroscopy, UV/Vis-spectroscopy and GPC measurement of the samples before and afterwards. No changes could be detected.

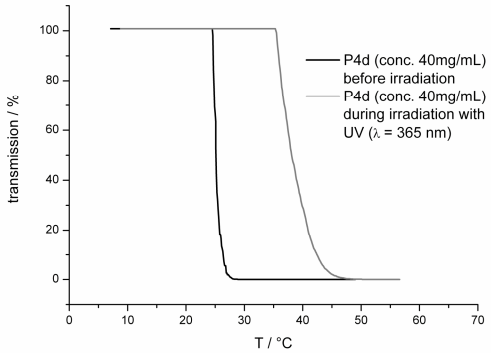


Figure 4. *LCST curves of the copolymer sample P4d before (black curve) and during irradiation (grey curve) with UV-light of 365 nm. The concentration was 40 mg/mL in Millipore water and the heating rate 1°C/min.*

However, the chromophores were instable in acidic media as the yellow colour of the polymer solution faded away immediately upon addition of concentrated hydrochloric acid. As an example the LCST curve of **P4d** before and during irradiation has been recorded (see figure 4).

For the copolymer series **P3**, we observed a higher LCST shift (4.3°C) for an amount of 4.0 mol % salicylideneaniline (**P3b**) compared to the LCST shift of 0.9°C for 1.0 mol% salicylideneaniline (**P3a**). However, for the copolymer series P4, the polymers with the lowest (**P4a**: 2.0 mol %) and the highest amount (**P4d**: 15.0 mol %) of incorporated salicylideneaniline showed the highest differences in LCST before and during irradiation. A maximum LCST-shift of 13.0°C was observed for copolymer **P4d**. Normally, a linear increase of the LCST-shift with increasing amount of chromophoric groups

would be expected. A reasonable explanation might be the statistical incorporation of the salicylideneaniline moieties in the polymer that may result in a neighboring effect of the photoswitchable units incorporated. A similar effect was already reported by our group for copolymer series based on poly(*N*-cyclopropyacrylamide) with azobenzene as a chromophoric group.[27]

Conclusion

In summary, we were able to prepare thermo and light-responsive copolymers containing different amounts of salicylideneaniline chromophores. The synthesis was based on a double polymer analogous reaction of poly(pentafluorophenyl acrylate) precursor polymers with the respective amines. Within the investigated polymers, the molecular weight distribution had been kept constant while maintaining a narrow molecular weight distribution, which was achieved by RAFT polymerization. The obtained copolymer series **P3** and **P4** exhibited a LCST in aqueous solution that depended strongly on the amount of incorporated chromophore. Furthermore, the isomerization of the salicylideneaniline chromophore in the copolymers, which was induced by irradiation with UV-light, had an influence on the LCST.

Higher LCST values up to 13.0°C were measured during irradiation and thus, in the temperature region between the LCST of the non-irradiated and the irradiated solution, a light controlled reversible solubility change was observed.

In our approach, the chromophore is not directly attached as a preformed chromophore to the polymer, but is rather build right at the polymer backbone, which opens the route to combine it with other immobilization chemistry, which may yield potential multi-functional and multi-responsive materials.

Acknowledgement

University of Mainz is gratefully acknowledged for financial support. S. Bonenberger and J. Podszuweit are acknowledged for support in the experimental work. P. J. Roth is acknowledged for photography.

References

(1) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163-249.

(2) Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *Macromol. Rapid Commun.* **2008**, *29*, 1172-1176.

(3) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2001**, *34*, 1391-1399.

(4) Okano, T.; Bae, Y. H.; Kim, S. W. *J. Controlled Release* **1990**, *11*, 255-265.

(5) Plate, N. A.; Valuev, I. L.; Valuev, L. I. *J. Biomater. Sci. Polym. Edn.* **2004**, *15*, 415-422.

(6) Eeckman, F; Amighi, K.; Moes, A. J. *Int. J. Pharm.* **2001**, *222*, 259-270.

(7) Shah, S. S.; Wertheim, J.; Wang, C. T.; Pitt, C. G. *J. Controlled Release* **1997**, *45*, 95-101.

(8) Li, W.; Tu, W.; Cao, D. *J. Appl. Polym. Sci.* **2009**, *111*, 701-708.

(9) Lutz, J.-F. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 3459-3470.

(10) Chen, J. P.; Yang, H. J.; Hoffman, A. S. *Biomaterials* **1990**, *11*, 625-630.

(11) Chen, H.; Liu, L.-H.; Wang, L.-S.; Ching, C.-B.; Yu, H.-W.; Yang, Y.-Y. *Adv. Funct. Mater.* **2008**, *18*, 95-102.

(12) Harada, A.; Johnin, K.; Kawamura, A.; Kono, K. *J. Polym. Sci. A. Polym. Chem.* **2007**, *45*, 5942-5948.

(13) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Chem. Commun.* **2009**, 2106-2108.

(14) Vogt, A. P.; Sumerlin, B. S. *Soft Matter* **2009**, in press.

(15) Li, D.; He, Q.; Yang, Y.; Moehwald, H.; Li, J. *Macromolecules* **2008**, *41*, 7254-7256.

(16) Magnusson, J. P.; Khan, A.; Pasparakis, G.; Saeed, A.; Wang, W.; Alexander, C. *J. Am. Chem. Soc.* **2008**, *130*, 10852-10853.

(17) Wang, G.; Tong, X; Zhao, Y. *Macromolecules* **2004**, *37*, 8911-8917.

(18) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(19) Kroeger, R.; Menzel, H.; Hallensleben, M. L. *Macromol. Chem. Phys.* **1994**, *195*, 2291-2298.

(20) Akiyama, H.; Tamaoki, N. *J. Polym. Sci. A: Polym. Chem.* **2004**, *42*, 5200-5214.

(21) Kungwatchakun, D.; Irie, M. *Makromol. Chem. Rapid. Commun.* **1988**, *9*, 243-246.

(22) Akiyama, H.; Tamaoki, N. *Macromolecules* **2007**, *40*, 5129-5132.

(23) Chunhua, L.; Fang, Z.; Zhaohui, Z.; Yuxing, P. *J. of Appl. Polmy. Sci.* **2007**, *107*, 2118-2125.

(24) Shimoboji, T.; Larenas, E.; Fowler, T.; Hoffman, A. S.; Stayton, P. S. *PNAS* **2002**, *99*, 16592-16596.

(25) Irie, M.; Kungwatchakun, D. *Proc. Japan Acad. Ser. B.* **1992**, *68*, 127-132.

(26) Luo, C.; Zuo, F.; Ding, X.; Zheng, Z.; Cheng, X.; Peng, Y. *J. of Appl. Polym. Sci.* **2008**, *107*, 2118-2125.

(27) Jochum, F. D.; Theato, P. *Polymer* **2009**, accepted, DOI: 10.1016/j.polymer.2009.05.041.

(28) Suzuki, T.; Kaneko, Y.; Arai, T. *Chem. Lett.* **2000**, *7*, 756-757.

(29) Anderson, D. G.; Wettermark, G. *J. Am. Chem. Soc.* **1965**, *87*, 1433-1438.

(30) Mitra, S.; Tamai, N. *Phys. Chem. Chem. Phys.* **2003**, *5*, 4647-4652.

(31) Ohshima, A.; Momotake, A.; Arai, T. *Chem. Lett.* **2005**, *34*, 1288-1289.

(32) Barbara, P. F.; Rentzepis, P. M.; Brus, L. E. *J. Am. Chem. Soc.* **1980**, *102*, 2786-2791.

(33) Thompson, B. C.; Abboud, K. A.; Reynolds, J. R.; Nakatani, K.; Audebert, P. *New J. Chem.* **2005**, *29*, 1128-1134.

(34) Ledbetter, J. W. *J. Phys. Chem.* **1966**, *70*, 2245-2249.

(35) Eberhardt, M.; Mruk, R.; Zentel, R.; Theato, P. *Eur. Polym. J.* **2005**, *41*, 1569-1575.

(36) Metz, N.; Theato, P. *Eur. Polym. J.* **2007**, *43*, 1202-1209.

(37) Nilles, K.; Theato, P. *Eur. Polym. J.* **2007**, *43*, 2901-2912.

(38) Theato, P. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 6677-6687.

(39) Eberhardt, M.; Theato, P. *Macromol. Rapid Commun.* **2005**, *26*, 1488-1493.

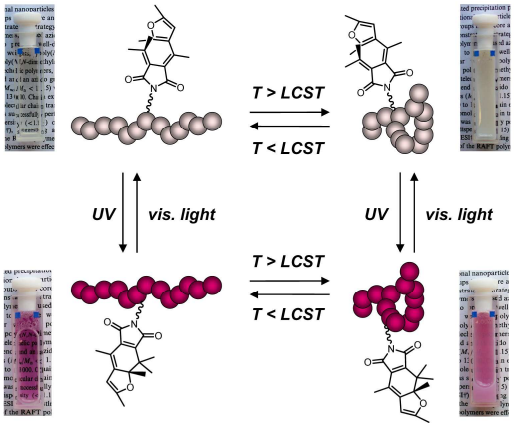
(40) Chong, Y. K.; Krstina, J.; Le, T. P.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2003**, *36*, 2256-2272.

PNIPAM Copolymers Containing Light-Responsive Chromophores: A Method Toward Molecular Logic Gates

*Florian D. Jochum, F. Romina Forst, Patrick Theato**

ABSTRACT

A series of thermo-responsive PNIPAM copolymers containing different amounts of fulgimide moieties have been synthesized via a polymer analogous reaction of poly(pentafluorophenyl acrylate). All copolymers were designed to exhibit a lower critical solution temperature (LCST) in water, which was only weakly dependent on the amount of incorporated chromophoric fulgimide groups. The copolymers showed a photocyclization of the fulgimide side groups upon irradiation with UV-light accompanied with a color change. The closed form of the chromophore had a halftime of 136 min for the visible reisomerization and did not affect the LCST of the polymer. This led to the realization of a logic "NOT A" for the fulgimide containing PNIPAM, while a corresponding azobenzene containing PNIPAM resulted in a different logic "A implies B".



Introduction

Molecular logic describes a concept of computation using chemical structures and has recently gained certain attention. The pioneering work of Adleman in 1994 was followed with various different concepts in material science.[1] However, today mainly small molecule based logical computations are investigated, with the most prominent based on photo-induced electron transfer (PET).[2] Only a small number of examples take advantage of polymers.[2,3] Especially when envisioning multi-input logic gates, functional polymer synthesis with an unprecedented precision and versatility is required, which has not been available until very recently. Polymer based logic gates have, however, the advantage of being much more processable and more versatile in the type of output and input signals than logic gates based on small molecules. In general, polymer based logic gates can be realized utilizing stimuli-responsive polymers that respond to at least to two different stimuli. Stimuli-responsive polymers have attracted great interest in academic and applied science recently. Most commonly, approaches take advantage of thermally induced, reversible phase transitions. In this context, polymers based on polyacrylamides found great interest as thermo-responsive polymers in aqueous solution. Many polyacrylamides, e.g. poly(*N*-isopropylacrylamide) (PNIPAM), feature a sharp transition behavior in water in response to changes of the temperature.[4] Accordingly, such polymers featuring a lower critical solution temperature (LCST) in water are expected to find application in many scientific areas.

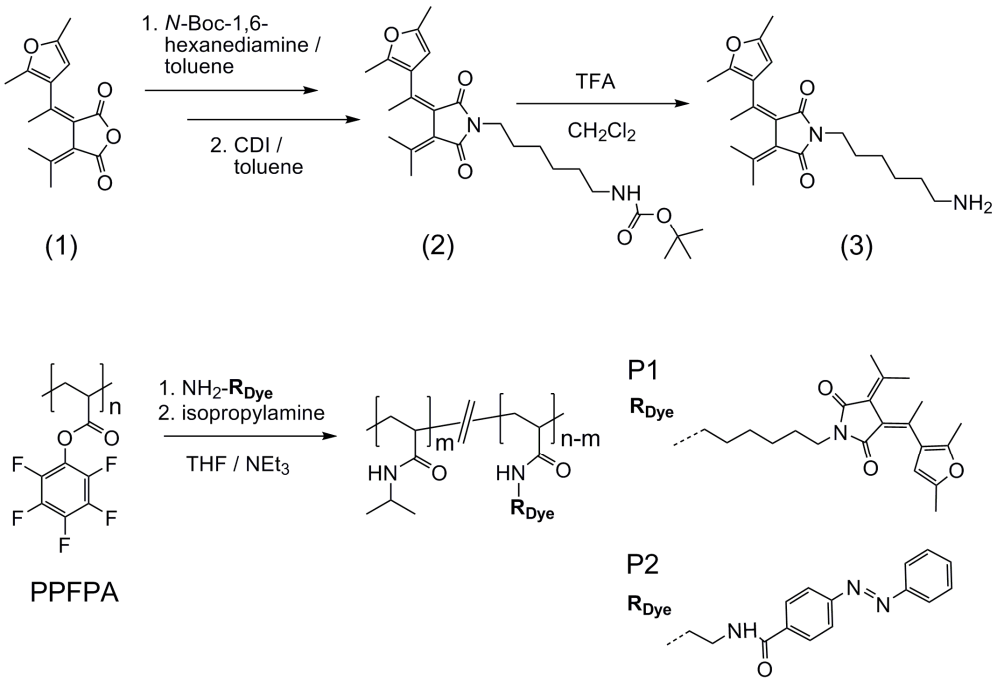
Besides polymers that are responsive to a single stimulus, recently research on polymers that show a responsive behavior to multiple stimuli has been intensified.[5] Other stimuli besides temperature can for example be pH,[6] ionic strength,[7] or light.[8,9] Very appealing and important for applications are polymers that are responsive to light and temperature and accordingly, there have been several reports on temperature-responsive copolymers that contain a light-responsive moiety, e.g. azobenzene,[10,11] spiropyrane[12] or salicylideneaniline[13] groups. Recently, we and other groups demonstrated that the LCST of thermo-responsive polymers containing light-responsive groups was dependant on the isomerization state of these functional groups and a light controlled solubility change was possible within a certain temperature range.[10a-d,11,13,14] Higher LCSTs were measured after photo-isomerisation of the chromophore side groups due to a change in the dipole moment or the polarity, which leads to a local increase of the whole polymer chain. Utilizing such changes in LCST for logic gates is a concept to be explored in more detail.

In the following, the synthesis of temperature- and light-responsive PNIPAM copolymers featuring a fulgimide as a novel photochromic group is reported. To the best of our knowledge, there exists no study in which the synthesis of water-soluble polymers containing fulgimides had already been reported. The synthesis of these polymers is based on the thorough investigated polymer analogous reaction (also called post-polymerization modification) of activated ester precursor polymers.[15] Fulgimides were reported to have photochromic properties similar to the parent fulgides, but the absorption bands show hypsochromic shifts. Further, these chromophores are known to isomerize upon irradiation with UV-light from the *E* to *Z* conformation or photocycle depending on the wavelength.[16] Based on the different classes of fulgide and fulgimide family compounds, many realistic and potential applications have been developed and suggested, e. g. actinometry, optical storage, optical data processing, and nonlinear optical materials, optical waveguides or optical switches.[16] Therefore, it is

synthetically a challenge to incorporate the fulgimide chromophore into a thermo-responsive polymer and to investigate their effect on the LCST of these compounds.

Experimental Section

All chemicals and solvents were commercially available and used as received unless otherwise stated. Tetrahydrofuran (THF) was distilled over sodium. Poly(pentafluorophenylacrylate) (**PPFPA**) with an molecular weight of M_n = 24900 g mol^{-1} and a molecular weight distribution of M_w/M_n = 1.36 was synthesized as described earlier.[13] (*E,Z*)-α-2,5-Dimethyl-3-furylethylidene (isopropylidene)-succinic anhydride (1) was synthesized according to the literature.[17] The detailed procedures for the synthesis of amino-functionalized fulgimide (3) and the conversion with **PPFPA** yielding the polymers **P1a-P1c** are give in the supporting information. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz FT-NMR spectrometer in deuterated solvents. ^{19}F NMR spectra were recorded on a Bruker 400 MHz FT-NMR spectrometer. Chemical shifts (δ) were given in ppm relative to TMS. Gel permeation chromatography (GPC) was used to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards. GPC measurements were performed in THF as solvent. The flow rate was 1 mL/min at 25°C. UV/Vis spectra were recorded on a Jasco V-630 photospectrometer. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer using an ATR unit. FD-masses were measured on a MAT 95 Finnigan mass spectrometer. Cloud points were determined in Millipore water at a concentration of 7.5 mg/mL and were observed by optical transmittance of a light beam (λ = 632 nm) through a 1 cm sample quartz cell. The measurements were



Scheme 1. *Synthesis of amino-functionalized fulgimide and synthesis of chromophore containing PNIPAM.*

performed in a Jasco V-630 photospectrometer with a Jasco ETC-717 peltier element. The intensities of the transmitted light were recorded versus the temperature of the sample cell. The heating rate was 1°C per minute. Irradiation experiments of the polymer solutions were performed in a 1 cm quartz cuvette using an Oriel Instruments 500 W mercury lamp (Output: 10mW/cm^2) with a 334 nm optical interference filter.

Results and Discussion

To enable a comparative study of stimuli-responsive copolymers featuring different chromophores, we have chosen to synthesize the respective polymers by a post-polymerization functionalization. As such, only the respective functional groups are introduced, while the degree of polymerization and the molecular weight distribution remain unchanged. This synthetic route is the only one that enables such a comparable study as any influence other than the chemical difference can be excluded. At first, an amino-functionalized fulgimide chromophore was synthesized capable to react with activated ester polymers. Therefore (*E,Z*)-α-2,5-dimethyl-3-furylethylidene (isopropylidene) succinic anhydride (**1**) was synthesized according to the literature[17] and further reacted with *N*-boc-1,6-hexanediamine to yield the boc-protected "half acid" isomer (see scheme 1). The obtained "half acids" derivates were cyclized with *N,N'*-carbodiimidazole (CDI) to yield the boc-protected fulgimide compound (**2**). In the next step, the protecting group was removed by treatment with trifluoroacetic acid resulting in the amino-functionalized compound (**3**).

Next, poly(pentafluorophenyl acrylate) **PPFPA** was synthesized by a reversible addition-fragmentation chain transfer (RAFT) polymerization as described recently.[13] The resulting precursor polymer had a molecular weight of M_n = 24.900 g/mol with a molecular weight distribution of M_w/M_n = 1.36. **PPFPA** was then subjected to a polymer analogous reaction with different amounts of the amino-

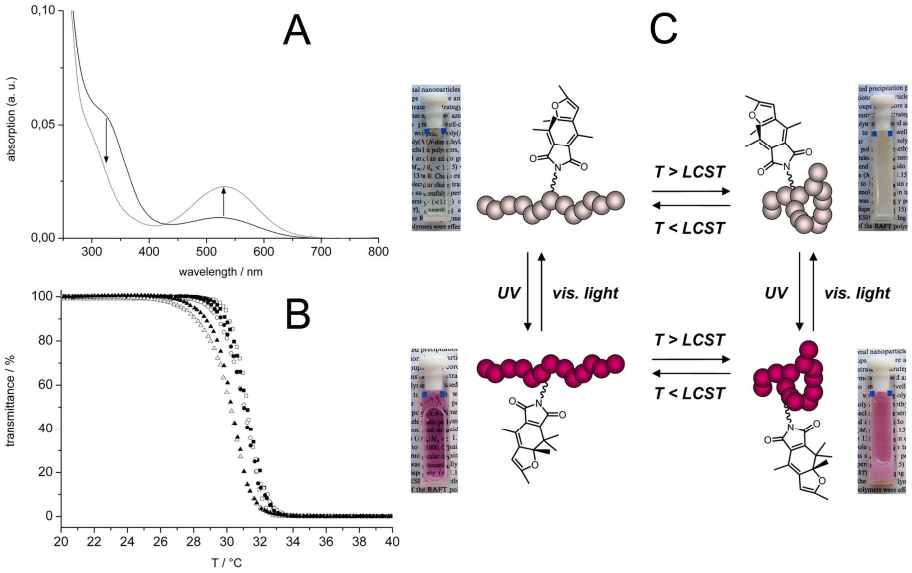


Figure 1. *A. UV/Vis spectra of the copolymer P1a (conc. 0.272 mg/mL in water) before (black line) and after irradiation (gray line) for 1h with UV-light (λ = 334 nm). B. LCST curves of the polymers P1a (rectangle), P1b (circle) and P1c (triangle) before (white symbols) and after irradiation (black symbols) with UV-light (λ = 334 nm) for 1 h. The concentration was 7.5 mg/mL in Millipore water. C. Overview of all possible states of the aqueous solution (7.5 mg/mL) of copolymer P1a.*

functionalized fulgimide (**3**) in tetrahydrofuran (THF) at room temperature (see scheme 1). After three hours of reaction, the remaining pentafluorophenyl ester groups were converted with an excess amount of isopropylamine yielding in a series of temperature-responsive PNIPAM copolymers containing different amounts of fulgimide side groups (**P1**). The resulting copolymers were purified by dialysis against methanol as well as precipitation into hexane to remove any low molecular weight impurities. All copolymers were analyzed by FT-IR-spectroscopy, GPC measurement, NMR-spectroscopy as well as UV/Vis spectroscopy. The quantitative conversion of the polymer analogous reaction was confirmed by IR-spectroscopy (see supporting information) as the activated ester polymer band at 1783 cm^{-1} of the **PPFPA** vanished completely and two amide bands at 1640 cm^{-1} and 1533 cm^{-1} of the copolymer **P1a** appeared.

After the polymer analogous reaction, the GPC trace of **P1a** (M_n = 8900 g/mol) was shifted to lower molecular weights in comparison to the GPC trace of the precursor **PPFPA** (M_n = 24900 g/mol) due to the reduction of molecular weight per repeating unit (see supporting information). Noteworthy, the molecular weight distribution M_w/M_n did not change significantly after the post-modification (see supporting information). The conversion was also followed by ^{1}H NMR spectroscopy (see supporting information). All characteristic proton signals from the side group of the polyacrylamide derivative (in $CDCl_3$: δ /ppm: 3.99, 1.11) and from the polymer backbone (in $CDCl_3$: δ /ppm: 2.69-1.34) were identified. Further, ^{19}F NMR spectroscopy was not able to detect a fluorine signal anymore, indicating a complete conversion of all pentafluorophenyl ester groups as well as a complete removal of pentafluorophenol salts formed during the reaction.

Table 1 summarizes the series of synthesized copolymers **P1** with varying contents of fulgimide ranging from calculated 1 mol% (**P1a**) to 5 mol% (**P1c**).

The composition of the copolymers was determined by UV/Vis spectroscopy based on the assumption that the absorption coefficient of polymer bound fulgimide was identical to that of the amino-functionalized dye (**3**) at 318 nm wavelength. A calibration curve of the dye was created by plotting the measured absorption maxima at different concentrations in methanol. Comparison to the absorption maxima at 318 nm of the copolymers solutions **P1a**, **P1b** and **P1c** in methanol yielded the amount of incorporated chromophore. The determined chromophore incorporation ratios of the copolymers were close to the theoretically calculated values (see table 1), demonstrating the efficiency of the polymer analogous reactions.

Table 1. *Composition and LCST values for the PNIPAM copolymer series containing various amounts of chromophores.*

chromophore = fulgimide				chromophore = azobenzene[11]			
Polymer	amount of fulgimide [mol%][a]	LCST before irradiation [°C] [b]	LCST after irradiation [°C] [b]	Polymer	amount of azobenzene [mol%][a]	LCST before irradiation [°C] [b]	LCST after irradiation [°C] [b]
P1a	1.0	31.2	31.1	P2a	1.0	29.3	29.9
P1b	2.7	31.1	31.0	P2b	2.0	29.1	29.6
P1c	3.6	30.1	30.2	P2c	3.9	20.5	23.7

a) measured by UV/Vis b) measured by turbidimetry

Additionally, the chromophoric behavior of the copolymers **P1** was thoroughly investigated by UV/Vis measurements. The color of the aqueous solutions ranged from colorless to slightly pink depending from the amount of incorporated fulgimide. However, all solutions turned deeply pink upon irradiation with UV-light with a wavelength of 334 nm (see figure 1). This phenomenon can be explained by the photocyclization of the fulgimide polymer side groups. The (*E*,*Z*)-fulgimide showed a photochemical conrotatory ring closure, which had already been studied for the single dye molecule in organic solvent.[16] Consequently, the chromophore bound at the polymer backbone should undergo the same reaction. The UV/Vis spectrum of the copolymer sample **P1a** is illustrated in figure 1. The absorption band at 530 nm increased strongly while the band at 325 nm was decreased after irradiation. The photocyclization reaction was fully reversible under exposure to visible light ($\lambda > 400$ nm) with a calculated halftime of $\tau_{1/2}$ = 136 min (see supporting information).

The LCSTs of the copolymer series were determined by turbidimetry, i.e., the optical transmittance of a light beam ($\lambda = 632$ nm) through the sample cell of the photospectrometer was monitored as a function of temperature. The concentration of all copolymer solutions **P1** was 7.5 mg/mL in Millipore water, and was choose as a suitable concentration for cloud point measurements based on recent other investigations.[14]

The cloud points were measured before and after irradiation with UV-light for 1 h ($\lambda = 334$ nm) with a heating rate of 1°C/min. The LCST was defined as the temperature at which a transmission of 50 % was observed. In general, the LCST of all copolymers was found to be only slightly decreased with increasing amount of hydrophobic chromophore but the influence was by far not as dramatic as for other dyes in PNIPAM copolymers as shown recently.[11,13] Noteworthy, the LCSTs of the aqueous solutions upon irradiation exhibited no detectable dependence on the content of incorporated fulgimide. In all cases, the same LCST-values were observed within the error range for LCST measurements after irradiation with UV-light. The LCSTs before and after irradiation are listed in table 1. Clearly, the LCSTs of the copolymers were not influenced by the photocyclization of the fulgimide side groups. The LCST curves of all copolymers **P1** before and after irradiation are shown in figure 1. Obviously, the

Table 2. *Logic truth table for polymers P1 and P2 with the following inputs and outputs. Input A: $I_A = 1$ (T = temperature at LCST before irradiation + 1°C), $I_A = 0$ (T < LCST)); Input B: $I_B = 1$ (irradiated sample), $I_B = 0$ (non-irradiated sample); Output: Out = 1 (soluble), Out = 0 (insoluble).*

chromophore = azobenzene			chromophore = fulgimide		
I_A	I_B	Out	I_A	I_B	Out
0	0	1	0	0	1
1	0	0	1	0	0
0	1	1	0	1	1
1	1	1	1	1	0
logic: “A implies B”			logic: “NOT A”		

structural change of the fulgimide side groups is too weak to increase or decrease the polarity of the polymer chain, even though the absorption maximum at 530 nm was increased.

In contrast to these results, we could demonstrate in recent publications, that the use of light-responsive chromophores showed an impact upon the LCST of PNIPAM-based copolymers.[11,13] The LCST values of thermo responsive PNIPAM copolymers containing azobenzene moieties are also listed in table 1. Clearly, azobenzene decreased the LCST of the PNIPAM copolymers stronger than the fulgimide. Further, higher LCSTs were measured upon irradiation due to the local increase of the dipole moment accompanied with the isomerisation of the chromophore side groups. In the case of the azobenzene based copolymers a maximum shift of the LCST of 3.2°C (**P2c**) was measured for an amount of 5.0 mol% chromophore, respectively (see table 1). The light controlled change of the LCST was fully reversible for whole azobenzene series (**P2**).[11] A similar trend for the LCST values was also found for PNIPAM based salicylideneaniline copolymers.[13]

Translating this information into terms of logic gates results in the fact that two different logic gates can be realized, even though the polymers have a similar composition and only differ in the nature of the chromophore used. A logic gate in general performs a logical operation on one or more logical inputs and produces a single logic output. As inputs the heating to a constant temperature (input A I_A) and the irradiation of the samples (input B I_B) can be used in the case of thermo-responsive polymers containing light-responsive moieties. Input $I_A = 0$ means that the sample is kept at a temperature below the LCST before irradiation, while $I_A = 1$ means that the sample is heated to a temperature one degree centigrade higher than LCST before irradiation. Further, $I_B = 0$ resembles the sample that has not been irradiated, while $I_B = 1$ corresponds to a sample that has been irradiated at the wavelength for the respective isomerization process of the chromophore. The output that can be read is whether the polymer sample is soluble (Out = 1) or insoluble (Out = 0). The translated logic data of the two polymer series **P1** and **P2** is compiled in table 2. It turns out that the azobenzene containing PNIPAM polymer **P2c** due to the differences in the LCST before and after irradiation acts as an "A implies B" logic, i. e. it is false if A is true but not B, otherwise true. In contrast, using a fulgimide as a chromophore results in a "NOT A" logic gate as the solubility (output) of these polymers are only dependent on one input the temperature (I_A) and the irradiation (I_B) does not effect the solubility behavior. In general these examples of thermo-responsive copolymers with different chromophores demonstrate a first step in a molecular computing through a different Boolean binary logic operation outputs and further more complex computations are expected in the future with polymers featuring responsive behavior depending on multiple (more than two) stimuli.

Conclusion

In summary, to realize the envisioned multi-input logic gates, we could successfully outline the synthesis of polymers with dual functionality and responsiveness in an unprecedented precision. We were able to prepare thermo responsive PNIPAM copolymers containing different amounts of fulgimide chromophores. The synthesis was based on a polymer analogous reaction of poly(pentafluorophenyl acrylate) precursor polymers with an amino-functionalized fulgimide. Within the investigated copolymers, the molecular weight distribution had been kept constant, which was achieved by RAFT polymerization. The obtained copolymer series P1 exhibited a LCST in aqueous solution that was hardly influenced by the amount of incorporated chromophore. Furthermore,

the photocyclization of the fulgimide, which was induced by irradiation with UV-light, did not effect the LCST at all. This led to the realization of a logic "NOT A", while the corresponding azobenzene containing PNIPAM resulted in a different logic "A implies B" and we thus could demonstrate for the first time that different polymer logic gates on the basis of stimuli-responsive polymers can be synthesized utilizing one reactive polymer precursor polymer by choosing appropriate constituents.

References

(1) Adleman, L. M. *Science* **1994**, *266*, 1021-1024.

(2) (2a) de Silva, A. P.; Uchiyama, S. *Nature Nanotech.* **2007**, *2*, 399-410; (2b) de Silva, A. P.; Uchiyama, S.; Vance, T. P.; Wannalerse, B. *Coord. Chem Rev.* **2007**, *251*, 1623-1632.

(3) (3a) Pasparakis, G.; Vamvakaki, M.; Krasnogor, N.; Alexander, C. *Soft Matter* **2009**, *20*, 3839-3841; (3b) Asoh, T.; Akashi, M. *Chem. Commun.* **2009**, 3548-3550; (3c) Uchiyama, S.; Kawai, N.; de Silva, A. P.; Iwai, K. *J. Am. Chem. Soc.* **2004**, *126*, 3032-3033.

(4) (4a) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163-249; (4b) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci*. **2004**, *29*, 1173-1222.

(5) (5a) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Chem. Commun.* **2009**, 2106-2108; (5b) Vogt, A. P.; Sumerlin, B. S. *Soft Matter* **2009**, *5*, 2347-2351.

(6) Li, D.; He, Q.; Yang, Y.; Moehwald, H.; Li, J. *Macromolecules* **2008**, *41*, 7254-7256.

(7) Magnusson, J. P.; Khan, A.; Pasparakis, G.; Saeed, A.; Wang, W.; Alexander, C. *J. Am. Chem. Soc*. **2008**, *130*, 10852-10853.

(8) Wang, G.; Tong, X.; Zhao, Y. *Macromolecules* **2004**, *37*, 8911-8917.

(9) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(10) (10a) Kroeger, R.; Menzel, H.; Hallensleben, M. L. *Macromol. Chem. Phys.* **1994**, *195*, 2291-2298; (10b) Akiyama, H.; Tamaoki, N. *J. Polym. Sci. A. Polym. Chem.* **2004**, *42*, 5200-5214; (10c) Kungwatchakun, D.; Irie, M. *Makromol. Chem. Rapid. Commun.* **1988**, *9*, 243-246; (10d) Akiyama, H.; Tamaoki, N. *Macromolecules* **2007**, *40*, 5129-5132; (10e) Chunhua, L.; Fang, Z.; Zhaohui, Z.; Yuxing, P. *J. of Appl. Polym. Sci.* **2007**, *107*, 2118-2125; (10f) Shimoboji, T.; Larenas, E.; Fowler, T.; Hoffman, A. S.; Stayton, P. S. *PNAS* **2002**, *99*, 16592-16596; (10g) Irie, M.; Kungwatchakun, D. *Proc. Japan Acad. Ser. B* **1992**, *68*, 127-132.

(11) Jochum, F. D.; Theato, P. *Polymer* **2009**, *50*, 3079-3085.

(12) Shiraishi, Y.; Miyamoto, R.; Hirai, T. *Org. Lett.* **2009**, *11*, 1571-1574.

(13) Jochum, F. D.; Theato, P. *Macromolecules* **2009**, *42*, 5941-5945.

(14) Jochum, F. D.; zur Borg, L.; Roth, P. J.; Theato, P. *Macromolecules* **2009**, *42*, 7854-7862.

(15) Theato, P. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 6677-6687.

(16) Crano, J. C.; Guglielmetti, R. J. *Organic Photochromic and Thermochromic Compounds* **2002**, *Vol. 1*, Kluwer Academic Publishers, 141-206.

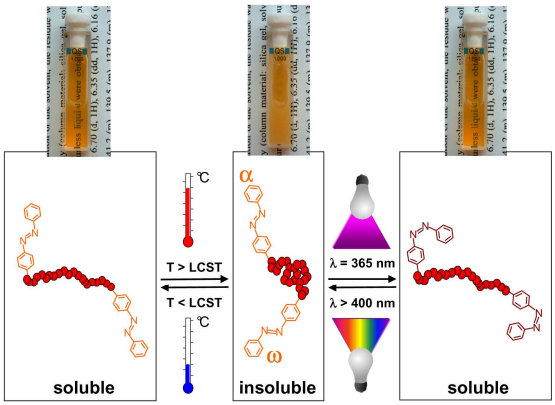
(17) Darcy, P. J.; Heller, H. G. Strydom, P. J.; Whittall, J. *J. Chem. Soc. Perkin Trans. 1*, **1981**, 202-205

Thermo- and Light-Responsive Polymers Containing Photoswitchable Azobenzene End Groups

*Florian D. Jochum, Lisa zur Borg, Peter J. Roth, Patrick Theato**

ABSTRACT

Telechelic thermo- and lightresponsive polymers based on poly(oligo(ethylene glycol) methyl ether methacrylate) P(OEGMA) with azobenzene functionalities at the end groups were synthesized. In a reversible addition fragmentation chain transfer (RAFT) polymerization using a functionalized chain transfer agent (CTA) containing a pentafluorophenyl (PFP) activated ester, oligo(ethylene glycol) methyl ether methacrylate (OEGMA, M_n ~ 300 g mol^{-1}) could successfully be polymerized with good control over molecular weight, very high conversions and narrow molecular weight distributions. Polymers derived from this CTA possessed an activated ester at the α-end of the polymer chain as well as a dithioester ω-terminus. The ω-dithioester group of each polymer chain could quantitatively be removed either with AIBN treatment or substituted with a PFP ester by using a modified diazo compound. As a consequence, a postmodification of the telechelic reactive end groups was possible through a polymer analogous reaction with amino-functionalized azobenzene. P(OEGMA) polymers containing azobenzene end groups showed a reversible light- and temperature controlled phase transition in water. Higher values for the lower critical solution temperature (LCST) were measured after irradiation of the aqueous polymer solutions due the higher polarity of *cis*-azobenzene. The LCST differences between irradiated and non irradiated solutions increased linearly upon the ratio of azobenzene units up to 4.3°C.



Introduction

Stimuli-responsive polymers, also called smart polymers, have been attracting great interest in academic and applied science recently. For instance, water-soluble polymers exhibiting a lower critical solution temperature (LCST) are potentially useful for several biomedical applications such as smart bioactive surfaces, selective bioseparation, phase separation immuno-assays or hyperthermia-induced drug delivery.[1-4] Many poly(*N*-alkylacrylamides) feature an LCST in aqueous solution[5-7] and so far, poly(*N*-isopropylacrylamide) (PNIPAM) has been the most studied thermosensitive polymer, because its LCST of 32 °C is close to the human body temperature.[1,8] Another class of thermally responsive systems is based on oligo(ethylene glycol) methyl ether methacrylates (OEGMA).[9-12] As many other methacrylate monomers, OEGMA can be polymerized under controlled radical polymerization methods yielding well defined materials. Polymers constructed from these PEG (macro)monomers exhibit fascinating solution properties in aqueous media. Depending on the molecular structure of their monomer units (i. e., nature of the polymerizable moiety, length of the PEG side chain, end group of the PEG side chain), the polymers can be insoluble in water, readily soluble up to 100 °C or even show a thermoresponsive behavior.

The area of polymers responsive to a single stimulus has been extended to polymers, which show a responsive behavior to multiple stimuli. Other stimuli besides temperature can for example be pH,[13] ionic strength[14] or light.[15,16] Of special importance seem polymers that are responsive to light and temperature and accordingly, there have been several reports on temperature-responsive poly(*N*-alkylacrylamide) copolymers, for example those containing light-responsive azobenzene in the side groups of the polymer chain.[17-22] Azobenzene groups are known to undergo a reversible isomerisation from *trans*- to *cis*-configuration upon irradiation.[23,24] In the excited *cis*-configuration, the higher dipole moment[25] leads to an increase of local polarity of the polymer chain, which causes an increase of the LCST.[17-22] As a result, these copolymers can be precipitated upon irradiation with UV-light within a certain temperature range. To the best of our knowledge, in most reports, the azobenzene chromophores were incorporated as side groups in the polymer backbone and not at the chain end. Upon increasing the number of azobenzene units, the photoinduced shift increased up to a certain value but decreased thereafter. These phenomena might be due to the variety in the number of azobenzene moieties and side group effects of the photofunctional units. Akiyama et al. demonstrated that photoisomerization of a single terminal unit of a polymer could also trigger a phase transition of a polymer chain.[26] They prepared an end-functionalized PNIPAM by atom transfer radical polymerization (ATRP) with an azobenzene derivative initiator. A linear increase of the LCST shifts with increasing amount of azobenzene located at the end group was found by this group which was in contrast to the transition behavior of azobenzene functionalized PNIPAM copolymers.

Telechelic polymers are polymers with the same functional group at both chain ends. Accordingly, it is synthetically challenge to introduce azobenzene functionalities at the both end groups of a polymer exhibiting an LCST. However, the investigation of the light controlled solubility change in the case of telechelic polymers and its potential application are as exciting. The linear chain of an LCST polymer such as P(OEGMA) then acts as a spacer between the α- and ω-end groups. A variety of methods to obtain end-group-functionalized polymers by controlled radical polymerization[27-33], especially those by reversible addition-fragmentation chain

transfer polymerization (RAFT)[34-39], have been reported. The RAFT polymerization[40] uses a dithioester chain transfer agent (CTA) and thus results in polymer chains that carry a CTA residue on their α-end and a dithioester at their ω-terminus. These dithioesters are very promising for a subsequent end-group functinalization.[36,37,39,41]

Herein, we want to extend the investigation of thermoresponsive polymers with azobenzene at the end group. In the following, the synthesis and characterization of thermoresponsive poly(oligo(ethylene glycol) methyl ether methacrylates) P(OEGMA) in two different molecular weights with either azobenzene at one end group or telechelic azobenzene moieties at both chain ends is described. All polymers were designed to exhibit an LCST in aqueous solution, which was known to be dependent on the amount of incorporated chromophoric azobenzene end groups, the molecular weight of the polymer and the isomerization state of the respective azobenzene end group. The synthesis is based on the RAFT polymerization of OEGMA-monomer (M_n ~ 300 g mol^{-1}) with 4 ethylene oxide units followed by post modification of the respective α- and ω-chain end groups. As CTA, pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA) will be used, which was described as a reasonable reagent for the synthesis of narrow distributed polymers with reactive terminus recently.[42-44] The LCST behavior of the resulting P(OEGMA) polymers with azobenzenes α- and ω-end group and the light controlled phase separation will be investigated in detail.

Experimental Section

Materials. All chemicals and solvents were commercially available and used as received unless otherwise stated. Oligo(ethylene glycol) methyl ether methacrylate (OEGMA) available as poly(ethylene glycol) methyl ether methacrylate from Aldrich (M_n ~ 300 g mol^{-1}) was distilled in high vacuum over 2,6-di-*tert*-butyl-*p*-cresol (DBPC) before use. Tetrahydrofuran (THF) and 1,4-dioxane were distilled over sodium. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at -7°C. As dialysis membranes Spectra/Por 3 (MWCO 3500) were used. 4-phenylthiocarbonylthio-4-cyanovaleric acid[45] and *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide[22] were synthesized as described in the literature.

Instrumentation. All ^1H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz FT-NMR spectrometer in deuterated solvents. ^{19}F NMR spectra were recorded on a Bruker 400 MHz FT-NMR spectrometer. Chemical shifts (δ) were given in ppm relative to TMS. Gel permeation chromatography (GPC) was performed in THF to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards. Calibration was done using polystyrene standards. UV/Vis spectra were recorded on a Jasco V-630 photospectrometer. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer using an ATR unit. Cloud points were determined in Millipore water at a concentration of 10 mg/mL and were observed by optical transmittance of a light beam (λ = 632 nm) through a 1 cm sample quartz cell. The measurements were performed in a Jasco V-630 photospectrometer with a Jasco ETC-717 peltier element. The intensities of the transmitted light were recorded versus the temperature of the sample cell. The heating rate was 1°C per minute. Irradiation experiments of the polymer solutions were performed with an Oriel Instruments 500 W mercury lamp with a 365 nm filter and an average output of 6.5 mW/cm^2 in a 1-cm diameter quartz cell (total volume 2.5 cm^3). Mass spectra were acquired by using a Shimadzu Axima CFR MALDI-TOF (Matrix Assisted Laser

Desorption/Ionization-Time of Flight) mass spectrometer equipped with a nitrogen laser delivering 3 ns laser pulses at 337 nm. Samples using α-cyano-4-hydroxycinnamic acid (CHCA) as matrix and KI as additive were prepared by dissolving the polymer in THF at a concentration of 10 g/L. First, an aliquot of 2 µL of the matrix solution (10 mg CHCA dissolved in 1 mL of 50% acetonitrile in 0.05 % trifluoroacetic acid) was placed on a multistage target plate. In the following, 2 µL of the polymer solution (10 mg of the polymer dissolved in 1 mL THF) and then 2 µL of the cationization agent solution (10 mg KI dissolved in 1 mL methanol) were added. The target plate was left to dry in air. The samples were analyzed with the operator manually and measured in positive reflectron ion mode. The laser was adjusted slightly above the ionization/desorption threshold.

Pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (*PFP*-CTA). 6.31 g (0.0226 mol) 4-phenylthiocarbonylthio-4-cyanovaleric acid and 4.81 g triethylamine (TEA) (0.0475 mol) were dissolved in 150 ml THF with magnetically stirring. The flask was sealed with a septum and 13.3 g (0.0475 mol) pentafluorophenyl trifluoracetate were added slowly through a syringe. The solution was stirred for 3 hours at room temperature and was then diluted with 150 ml of dichloromethane. Afterwards the solution was transferred into a separating funnel and washed three times with 50 mL of water. The organic phase was separated, dried with sodium sulfate and after filtration, it was concentrated in vacuum. The product was purified by column chromatography (column material: silica gel, solvent: petroleum ether / ethyl acetate 5:1) and finally dried in high vacuum overnight. 7.62 g (0.0171 mol – 76 %) of pure red colored pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) was obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 7.91 (d, 2H), 7.57 (t, 1H), 7.39 (t, 2H), 3.08-3.01 (m, 2H), 2.80-2.70 (m, 1H), 2.59-2.49 (m, 1H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$): δ /ppm: 219.7, 167.8, 144.3, 142.7, 139.4, 136.3, 134.4, 133.2, 128.6, 126.7, 118.2, 45.5, 32.9, 29.1, 24.2; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.28 (t, 2F), -157.69 (t, 1F), -152.88 (d, 2F); FT-IR (ATR-mode): 2927 cm^{-1} (C-H valence band), 1785 cm^{-1} (C=O reactive ester band), 1516 cm^{-1} (PFP C=C aromatic band)

Bis(pentafluorophenyl)-4,4'-azobis(4-cyano-valerate) (*PFP*-ACV). 3.85 g (0.0137 mol) 4,4'-azobis(4-cyano valeric acid) and 5.55 g (0.0549 mol) TEA were dissolved in 150 ml dry THF. 15.38 g (0.0549 mol) pentafluorophenyl trifluoracetate were added slowly through a dropping funnel and the solution was allowed to stir for 5 hours at room temperature. Afterwards, 150 ml dichloromethane were added, the solution was transferred into a shaking funnel and was washed three times with 50 ml water. The organic phase was separated, dried with sodium sulfate and after filtration it was concentrated in light vacuum at 30°C. The raw product was dissolved in 20 ml dichloromethane and precipitated into cold hexane. After filtration and washing with cold hexane, the colorless solid was dried in vacuum at room temperature. 6.54 g (0.0107 mol – 78 %) of bis(pentafluorophenyl)-4,4'-azobis(4-cyanovalerate) was obtained and stored at -7°C. ^{1}H NMR ($CDCl_3$): δ /ppm: 3.00-2.48 (m, 8H), 1.78 (s, 3H), 1.73 (s, 3H); ^{13}C NMR ($CDCl_3$): δ /ppm: 167.5, 142.6, 141.4, 139.5, 138.1, 136.2, 117.0, 71.7, 32.7, 28.3, 23.9; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.20 (t, 4F), -157.56 (t, 2F), -152,88 (d, 4F); FT-IR (ATR-mode): 2929 cm^{-1} (C-H valence band), 1786 cm^{-1} (C=O reactive ester band), 1517 cm^{-1} (PFP C=C aromatic band)

General RAFT polymerization procedure (P1a and P1b). Both RAFT polymerisations followed the same procedure. *PFP*-CTA, AIBN and freshly distilled OEGMA-monomer were placed into a Schlenk-tube with varying ratios (see table 1). After addition of 20 mL dry 1,4-dioxane, four freeze-pump-thaw cycles were performed to degas the

solution. The flask was filled with argon, immersed into a preheated oil bath of 80 °C and stirred overnight. After cooling down to room temperature, the polymer was isolated by precipitation in hexane. The crude polymer was dissolved in THF, precipitated again three times into hexane, centrifuged and finally dried in vacuum. Usually, 90% of red colored viscous *PFP*-P(OEGMA) (P1a and P1b) were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 7.81 (d), 7.46 (t), 7.30 (t), 4.03 (br s), 3.83 (br s), 3.59 (br d), 3.49 (br t), 3.39 (s), 3.32 (s), 3.25 (s), 3.08 (s), 3.04 (s), 2.81 (br s), 2.18 (br s), 1.82 (br m), 1.22 (br m), 0.90 (br d); ^{13}C NMR ($CDCl_3$): δ /ppm: 177.0, 128.1, 126.4, 71.6, 70.3, 68.2, 63.6, 58.7, 54.1, 44.4, 18.3, 16.1; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.5 (t, 2F), -158.1 (m, 1F), -153.0 (d, 2F); FT-IR (ATR-mode): 2875 cm^{-1} (C-H valence band), 1789 cm^{-1} (C=O reactive ester band), 1727 cm^{-1} (C=O ester band), 1519 cm^{-1} (PFP C=C aromatic band), 1250 cm^{-1} (C-O ester band), 1097 cm^{-1} (C-O ether band)

General procedure for dithioester removal of *PFP*-P(OEGMA) polymers with AIBN (P2a and P2b). In a typical run, *PFP*-P(OEGMA) polymer (P1a and P1b) containing a dithioester end group at the ω-position and 30 equiv of AIBN were dissolved in a Schlenk-tube in dry dioxane. 18 mL of dioxane was used for 5 mmol of diazo component. After addition of AIBN, the Schlenk-tube was sealed with a septum, placed immediately into a preheated oil bath at 80°C and stirred for 3.5 h. After cooling down to room temperature, the polymer was isolated by precipitation into hexane. The crude polymer was dissolved in THF, precipitated again three times into hexane, centrifuged and finally dried in vacuum. Usually, between 76-85 % of colorless and viscous polymers (P2a and P2b) were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 4.04 (br s), 3.83 (br s), 3.62 (br d), 3.50 (br t), 3.40 (s), 3.33 (s), 3.26 (s), 3.10 (s), 3.05 (s), 2.83 (br s), 2.02 (s), 1.86 (br m), 1.22 (br m), 0.92 (br d); ^{13}C NMR ($CDCl_3$): δ /ppm: 177.0, 71.7, 70.3, 68.3, 63.6, 58.8, 54.3, 44.6, 18.3, 16.1; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.4 (t, 2F), -158.0 (m, 1F), -153.0 (d, 2F)

General procedure for the synthesis of P(OEGMA) polymers with PFP-ester at the ω– and α-position (P3a and P3b). In a typical run, *PFP*-P(OEGMA) polymer (P1a and P1b) containing a dithioester end group at the ω-position and 30 equiv of *PFP*-ACV were dissolved in a Schlenk-tube in dry dioxane. 18 mL of dioxane was used for 5 mmol of diazo component. After addition of the *PFP*-ACV, the Schlenk-tube was sealed with a septum, placed immediately in a preheated oil bath at 80 °C and stirred for 3.5 h. After cooling down to room temperature, the polymer was isolated by precipitation in hexane. The crude polymer was dissolved in THF, precipitated again three times into hexane, centrifuged and finally dried in vacuum. Usually, between 75-81 % of colorless and viscous polymers (P3a and P3b) were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 4.04 (br s), 3.68 (s), 3.61 (br d), 3.52 (br t), 3.40 (s), 3.35 (s), 3.26 (s), 3.20 (s), 3.10 (s), 2.83 (br s), 1.83 (br m), 1.22 (br m), 0.91 (br d); ^{13}C NMR ($CDCl_3$): δ /ppm: 177.0, 71.7, 70.3, 68.3, 63.6, 58.8, 54.1, 44.7, 18.4, 16.5; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.4 (t, 4F), -158.0 (m, 2F), -153.0 (d, 4F)

General procedure for the synthesis of P(OEGMA) polymers with azobenzene at the α-position (P4a and P4b). P(OEGMA) polymers containing azobenzene end groups in α-position were obtained by reaction of the polymers P2a and P2b with *N*-(2-aminoethyl)-4-(2-phenyl-diazenyl)benzamide. Therefore, P2a and P2b were dissolved with 5 equiv *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide and 1 equiv TEA in dry THF. 10 ml THF was used for 0.2 mmol of the azobenzene compound. The solutions were stirred for 12 h under nitrogen atmosphere. Then, the

solvent was evaporated in vacuum and the residues were suspended in cold water. After filtration of the insoluble excess *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide, the orange colored filtrates were filled into dialysis membranes and dialyzed against diluted aqueous ethanol (30-60 %) for 2 days to remove the remaining *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide and the pentafluorophenol salts. Every 6 hours the solvent was refreshed. Afterwards, the contents of the membranes were transferred into a flask and concentrated in vacuum. The polymer residues were redissolved in THF and isolated by precipitation into hexane. After centrifugation and drying in vacuum, usually between 71-76 % of orange colored viscous polymers (P4a and P4b) were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 7.94 (m), 7.72 (br s), 7.47 (d), 6.81 (br s), 4.04 (br s), 3.83 (br s), 3.61 (br d), 3.51 (br t), 3.33 (s), 3.10 (s), 2.33 (br s), 1.83 (br m), 1.22 (br m), 0.91 (br d); FT-IR (ATR-mode): 2875 cm^{-1} (C-H valence band), 1727 cm^{-1} (C=O ester band), 1657 cm^{-1} (C=O amide band I), 1542 cm^{-1} (C=O amide band II), 1250 cm^{-1} (C-O ester band), 1097 cm^{-1} (C-O ether band)

General procedure for the synthesis of P(OEGMA) polymers with telechelic azobenzene functionality (P5a and P5b). P(OEGMA) polymers containing azobenzene end groups in α- and ω–position were obtained by reaction of the polymers P3a and P3b with *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide. Therefore, P3a and P3b were dissolved with 10 equiv of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide and 2 equiv TEA in dry THF. 10 ml THF was used for 0.2 mmol of the azobenzene compound. The solutions were stirred for 12 h under nitrogen atmosphere. In the following the solvents were evaporated in vacuum and the residues were suspended in cold water. After filtration of the insoluble excess *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide, the orange colored filtrates were filled into dialysis membranes and dialyzed against diluted aqueous ethanol (30-60 %) for 2 days to remove the remaining *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)-benzamide and the pentafluorophenol salts. Every 6 hours the solvent was refreshed. Afterwards, the contents of the membranes were transferred into a flask and concentrated in vacuum. The polymer residues were redissolved in THF and isolated by precipitation into hexane. After centrifugation and drying in vacuum, usually between 60-69 % of orange colored viscous polymers (P5a and P5b) were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 7.94 (m), 7.72 (br s), 7.47 (d), 6.81 (br s), 4.04 (br s), 3.83 (br s), 3.61 (br d), 3.51 (br t), 3.33 (s), 3.10 (s), 2.33 (br s), 1.83 (br m), 1.22 (br m), 0.91 (br d); FT-IR (ATR-mode): 2875 cm^{-1} (C-H valence band), 1727 cm^{-1} (C=O ester band), 1657 cm^{-1} (C=O amide band I), 1542 cm^{-1} (C=O amide band II), 1250 cm^{-1} (C-O ester band), 1097 cm^{-1} (C-O ether band)

Results and Discussion

The scope of the present study was the preparation of telechelic thermoresponsive P(OEGMA) polymers that possessed azobenzene moieties either on one or on both end groups of the chain. In order to reach this goal, several individual techniques to prepare α- or ω-end-group functionalized polymers were combined. As former investigations had shown, the pentafluorophenyl (PFP) ester is a very versatile and reactive functionality. Accordingly, pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (*PFP*-CTA) was obtained in a slightly different way as already previously reported by our group[42]. For that, first 4-phenylthiocarbonylthio-4-cyanovaleric acid was synthesized as described in the literature[45] and treated with pentafluorophenyl trifluoracetate. The final product was obtained after purification through column chromatography in 76 % yield.

Synthesis of *PFP*-P(OEGMA) polymers (see scheme 1, route A). *PFP*-CTA was used as a chain

transfer reagent for RAFT polymerization of oligo(ethylene glycol) methyl ether methacrylate (OEGMA). The two resulting red colored viscous polymers *PFP*-P(OEGMA) **P1a** and **P1b** were analyzed by NMR-spectroscopy, FT-IR spectroscopy, UV/Vis-spectroscopy and GPC. ^{1}H-NMR polymers (see figure 1) showed the characteristic aromatic signals of the dithioester ω-end group at 7.81 ppm, 7.46 ppm and 7.30 ppm.

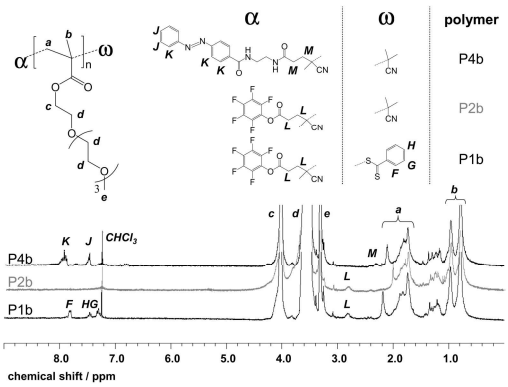


Figure 1. *^{1}H NMR spectra measured in deuterated chloroform of the polymers P1b, P2b and P4b.*

^{19}F NMR spectroscopy was performed to analyze the end group of both polymers. The fluorine signals at a chemical shift of -162.5 ppm, -158.1 ppm and -153.0 ppm of the PFP-ester proved the existence of reactive α-terminus. Additionally, the FT-IR spectrum (see figure 2) showed the polyacrylate band at 1727 cm^{-1} and also the characteristic bands at 1789 cm^{-1} and 1519 cm^{-1} of the PFP-ester end group. The UV/Vis spectrum, as illustrated in figure 3, showed the characteristic absorption bands of the dithioester at 302 nm and 503 nm.[41]

The molecular weight of the polymers **P1a** and **P1b** was determined by GPC and in addition by end group analysis using ^{1}H NMR spectroscopy. The values for the molecular weights are listed in table 1. For the calculation of the molecular weight by ^{1}H NMR spectroscopy, the ratio of the aromatic dithioester signals (7.81 ppm, 7.46 ppm and 7.30 ppm) and the single signal of the methoxy protons (3.32 ppm) was calculated.

The values for the number average of the molecular mass M_n determined by GPC (**P1a**: M_n = 8600 g mol^{-1}, **P1b**: M_n = 5000 g mol^{-1}) were slightly higher than the calculated values and the ones measured by NMR (**P1a**: M_n = 7900 g mol^{-1}, **P1b**: M_n = 3800 g mol^{-1}). The reason for this divergence was the calibration of the GPC with respect to polystyrene standards. However, the polydispersity M_w/M_n of 1.15 and 1.18 indicated a narrow molecular weight distribution for both *PFP*-P(OEGMA) polymer samples.

As an example, the GPC curve of the *PFP*-P(OEGMA) polymer **P1b** with the UV/Vis detector set to the absorption maximum of the dithioester (302 nm) is illustrated in figure 4. Altogether, two different *PFP*-(POEGMA) polymers with different molecular weights, that means two different ratios

Table 1. *Characteristic data for the P(OEGMA) polymers P1a and P1b.*

Polymer	*PFP*-CTA	AIBN	OEGMA-monomer	M_n (calc.)	M_n (GPC)	M_n (NMR)	M_w/M_n (GPC)
P1a	456 mg (1.025 * 10^{-3} mol)	21 mg (1.280 * 10^{-4} mol)	7.38 g (0.0265 mol)	7800 g mol^{-1}	8600 g mol^{-1}	7900 g mol^{-1}	1.18
P1b	826 mg (1.856 * 10^{-3} mol)	38 mg (2.317 * 10^{-4} mol)	6.40 g (0.0230 mol)	3700 g mol^{-1}	5000 g mol^{-1}	3800 g mol^{-1}	1.15

between the OEGMA-repeating units and the α- and ω-end group were obtained in a narrow molecular weight distribution.

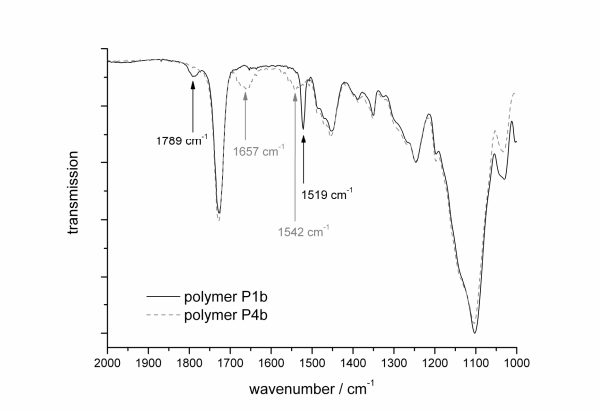


Figure 2. *FT-IR spectra of the polymers P1b (black solid line) and P4b (gray dashed line).*

Dithioester removal of *PFP*-P(OEGMA) polymers with AIBN (see scheme 1, route B). Next the *PFP*-P(OEGMA) polymers **P1a** and **P1b** containing a reactive ester functionality at the α- and a dithioester at the ω-endgroup were post modified. The removal of the dithioester was crucial to prevent the formation of disulfide coupling reactions which might occur during a polymer analogous reaction of the α-end group.

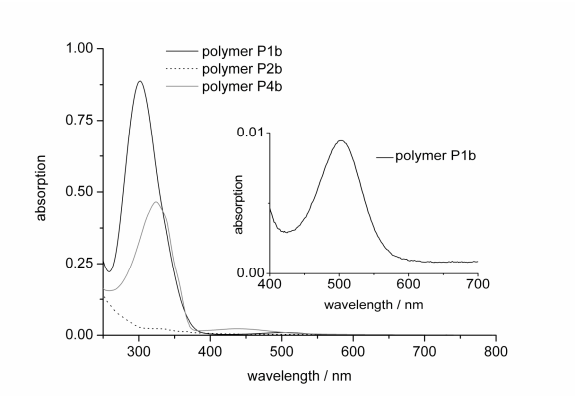


Figure 3. *UV/Vis spectra of the polymers P1b (black solid curve, conc. 0.400 mg/mL acetonitrile), P2b (black dotted curve, conc. 0.349 mg/mL acetonitrile) and P4b (grey solid curve, conc. 0.125 mg/mL acetonitrile).*

The dithioester of the *PFP*-P(OEGMA) polymers **P1a** and **P1b** was removed with an excess amount of AIBN in a slightly different procedure as reported by Perrier at al.[37] For that, two samples of the **P1a** and **P1b** were stirred with 30 equiv of AIBN for 3.5 hours at a constant temperature of 80 °C. A preheated oil bath with a constant temperature of 80 °C was crucial for a successful reaction and to inhibit high molecular disulfide formation. The obtained polymers **P2a** and **P2b** were analyzed by ^{1}H NMR, ^{19}F NMR, UV/Vis spectroscopy as well as GPC measurements. The ^{1}H NMR as illustrated in figure 1 showed the complete removal of dithioester group as the aromatic protons of the ω-endgroup at 7.81 ppm, 7.46 ppm and 7.30 ppm were vanished completely. The UV/Vis spectrum (see figure 3) did not show the dithioester absorption band at 302 nm any longer. Additionally, samples of the polymers **P2a** and **P2b** were analyzed by ^{19}F NMR to exclude any decomposition of the α-functionality. The PFP-ester was not harmed by this treatment, as PFP-esters are stable toward radicals and elevated temperature in the absence of nucleophiles. Clearly, the ^{19}F NMR spectra of the polymers obtained after AIBN treatment still showed the typical PFP ester signals.

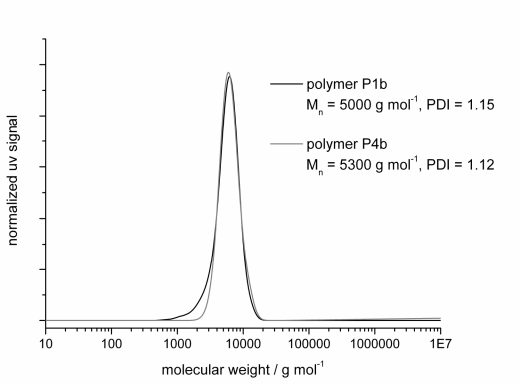


Figure 4. *Normalized GPC elugrams of P1b (black line) measured at 302 nm and P4b (gray line) measured at 323 nm.*

The GPC curve of the polymers **P2a** and **P2b** showed no shoulder at twice the molecular weight indicating that no disulfide coupling reaction had occurred (see supporting information). The molecular weights and the molecular weight distribution were still the same as measured for the polymers **P1a** and **P1b**.

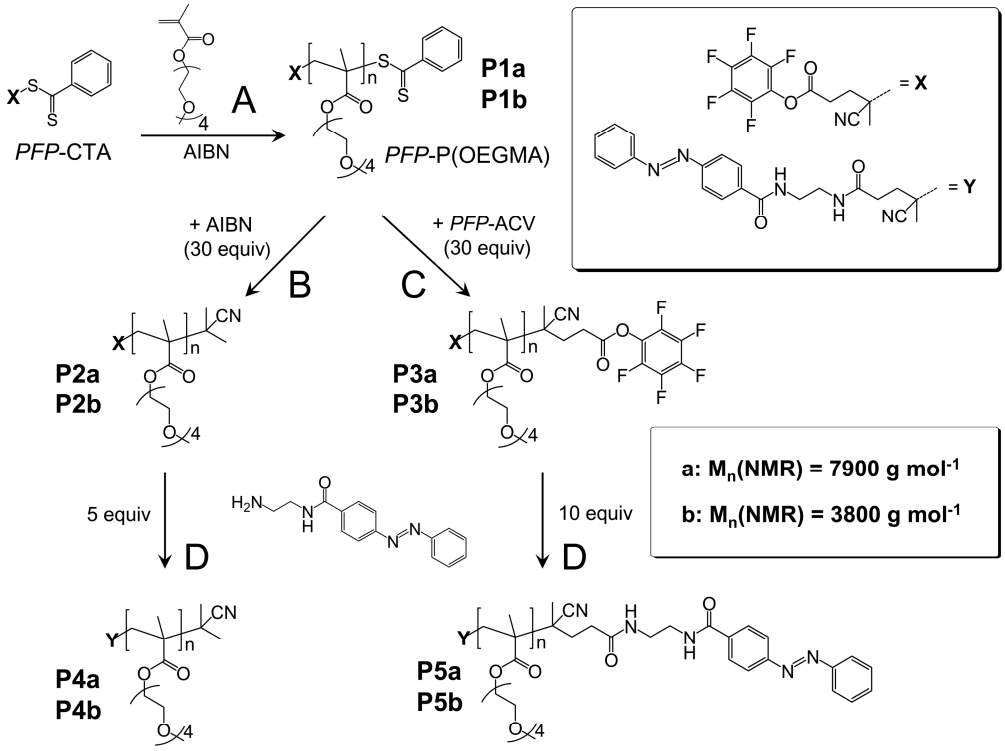
Synthesis of P(OEGMA) polymers with PFP-ester at the ω– and α-position (see scheme 1, route C). To introduce a pentafluorophenyl ester end group at the ω-position, the dithioester end group of the polymers had to be exchanged in a reaction described in detail by the group of Theato.[42] This method enabled the synthesis of P(OEGMA) polymers with telechelic PFP-ester functionality. Quite similar to the conditions reported there, two samples of the dithioester-terminated *PFP*-P(OEGMA) polymers **P1a** and **P1b** were treated with an excess amount bis(pentafluorophenyl)-4,4'-azobis(4-cyano-valerate) (*PFP*-ACV). The *PFP*-ACV was therefore previously synthesised by activating the commercial available 4,4'-azobis(4-cyano valeric acid) with pentafluorophenyl trifluoracetate. In analogy to the procedure for the dithioester removal with AIBN, the both *PFP*-P(OEGMA) polymers **P1a** and **P1b** were stirred with 30 *PFP*-ACV equiv 3.5 hours at a constant temperature of 80 °C.

Full conversion of the end group modification was confirmed for the polymers **P3a** and **P3b** again by ^{1}H NMR, ^{19}F NMR and UV/Vis spectroscopy and GPC measurements. The aromatic signals of the dithioester ω-end group in the proton NMR spectrum had completely vanished while the typical fluorine signals of the PFP ester in the fluorine spectra were still present.

The UV/Vis spectrum did not show anymore the characteristic absorption band at 302 nm, indicating the formation of telechelic polymers with activated ester functionality at both sides of the chain.

Again, the GPC curve of the polymers **P3a** and **P3b** showed no shoulder at twice the molecular weight, indicating that no disulfide coupling reaction had occurred.

The molecular weights and the molecular weight distribution were still the same as measured for the polymers **P1a** and **P1b**.



Scheme 1. *Overview of reactions described in detail in the text.*

Synthesis of thermo- and lightresponsive polymers with azobenzene end group functionality (see scheme 1, route D). In order to address the terminal activated ester either on one side or on both sides of the polymer chains, four samples of the post-modified *PFP*-P(OEGMA) polymers **P2a-b** and **P3a-b** respectively, were reacted with an excess amount of aliphatic amino-functionalized azobenzene. For that, *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide was synthesized in a reaction according to a previous publication.[22] In a typical polymer analogous reaction the polymers were stirred in THF solution with 5 equiv of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide for the polymers **P2a-b** with one PFP-ester at the α-end and 10 equiv of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide for the polymers **P3a-b** with telechelic PFP-ester functionality. All polymer samples were purified through filtration and dialysis against diluted aqueous ethanol, in order to remove the remaining excess *N*-(2-aminoethyl)-4-(2-phenyldiazenyl) benzamide and the formed pentafluorophenol salts. The obtained polymers **P4a-b** and **P5a-b** were all orange colored in different intensity, and a GPC measurement with the UV/Vis detector set to the absorption maximum of azobenzene (323 nm) indicated that azobenzene was successfully attached to the polymer chain end and any excess *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)benzamide had been completely removed by dialysis (see figure 4). The GPC curve of the azobenzene-functionalized polymers showed no shoulder at twice the molecular weights, however, fractions with a lower molecular weight than 3500 g/mol were totally removed through the dialysis. The molecular weight and the molecular weight distribution M_w/M_n had not changed significantly, indicating that no disulfide coupling had occurred during the dithioester removal reaction. MALDI-TOF mass spectrometry was used in addition to determine the molecular weight in the case of the polymer **P4b** (see supporting information). Noteworthy, the average molecular weight of 4500 g mol^{-1} was in the range between the mass values as measured by ^{1}H NMR (M_n = 3800 g mol^{-1}) and GPC (M_n = 5300 g mol^{-1}). Additionally, NMR spectroscopy was performed to analyze the synthesized **P4a-b** and **P5a-b** polymers. The ^{1}H NMR spectra (see figure 1) showed the characteristic signals of the aromatic azobenzene protons at 7.94 ppm and 7.47 ppm. Further, ^{19}F NMR spectroscopy was not able to detect any fluorine signals, indicating a complete conversion of all pentafluorophenyl ester end groups. The FT-IR spectroscopy confirmed the successful modification of the end groups as the bands at 1789 cm^{-1} and 1519 cm^{-1} of the PFP-ester end group had totally vanished and two amide bands of the azobenzene end group at 1657 cm^{-1} and 1542 cm^{-1} had appeared (see figure 2). In the UV/Vis spectrum of the **P4b** as illustrated in figure 3 the characteristic absorption band at 323 nm of the azobenzene chromophore could be assigned.

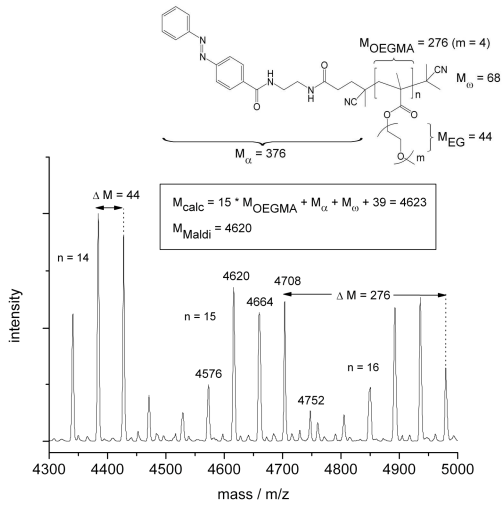


Figure 5. *Section of the MALDI-TOF spectrum of polymer P4b, demonstrating the high end-group functionalization efficiency.*

Figure 5 shows exemplary a section of the MALDI-TOF mass spectrum of **P4b** in order to demonstrate the high efficiency during the end-

group transformation (full MALDI-TOF spectrum, see supporting information).

Clearly two statistical distributions can be observed: a) distribution of the ethylene glycol side chain length, with the typical peak separation of 44 (equivalent to the mass of an ethylene glycol unit) and b) the distribution of the main chain repeating units, with the typical peak separation of 276 (equivalent to the mass of a repeating unit with 4 ethylene glycol units in the side chain). All peaks can clearly be assigned to the polymer exhibiting the proposed end-groups. For example, polymer **P4b** with n = 15 repeating units in the back bone and m = 4 side chain ethylene glycol units gives a theoretical mass of 4623, which can be clearly assigned to the peak located at 4620 in the MALDI-TOF.

Each of the four polymers **P4a-b** and **P5a-b** differed in the amount of incorporated azobenzene chromophores as the polymers had a varying ratio between the end groups and the molecular weight. The conversion of the end groups of the respective polymers was determined by UV/Vis spectroscopy assuming that every polymer chain end had precisely one reactive ester group for the polymers **P2a-b** and two reactive ester groups in the case of the telechelic polymers **P3a-b**.

Further, the ratio between end group and the repeating units was used from the molecular weight values measured by ^{1}H-NMR as listed in table 1. The absorption coefficient of the polymer bound azobenzene was assumed to be identical to that of free *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)-benz-amide at 323 nm. For that, a calibration curve of *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)-benz-amide was calculated by measuring the absorption maximum at different concentrations in ethanol. Comparison to the absorption maxima at 323 nm of the different polymer solutions in ethanol yielded the incorporated amount of chromophore (see table 2). Altogether, the values showed that the polymer

Table 2. *Composition and conversions for the PFP-ester end groups of the P(OEGMA)-polymers.*

Polymer	amount of azo-benzene [mol %] *(calculated)*	amount of azo-benzene [mol %] *(measured by UV/Vis)*	conversion of the end groups [%]
P4a	3.8	3.5	93
P5a	7.6	6.1	80
P4b	7.9	7.8	98
P5b	16.8	12.5	79

analogous reaction of the PFP-ester end functionalized polymers was performed successfully with high conversions. For the polymers **P4a** and **P4b** with one functional end group at the α-terminus, an almost quantitative conversion of the PFP-ester (≥ 93 %) was measured. However, the values for the polymers P5a and P5b with telechelic azobenzene functional group were lower and in the range between 79 % and 80 %. These values with an estimated error of 10 % represent reasonable data which we could also observe in a recent publication.[42]

As the P(OEGMA) polymers **P4a-b** and **P5a-b** containing azobenzene chromophores at the chain end were designed to exhibit a light and temperature controlled phase separation in aqueous solution, cloud point measurements were performed to determine the LCST. The cloud points were measured before and after irradiation with UV-light (λ = 365 nm) for 1 hour. The LCST was defined as the temperature at which a transmission of 50 % was observed. However the LCST is dependent on the concentration of the polymer solution, the molecular weight of the polymer and effects of salts. Therefore a defined concentration of 10 mg/mL polymer in Millipore water was chosen, which had already been demonstrated in a recent publication as a suitable concentration to determine the LCST of P(OEGMA) polymers.[9] In all measurements, a slight temperature hysteresis shifted to lower LCST-values during the cooling

period was observed. Typically, the LCST-values of the heating cycle were 0.5 °C higher than the values of the cooling cycle. The LCSTs values for the heating period are listed in table 3.

Table 3. *Composition and LCST values for the P(OEGMA) polymers.*

P.	amount of azo-benzene [mol %] *(measured by UV/Vis)*	LCST before irradiation [°C]	LCST after irradiation [°C]	Δ LCST [°C]
P4a	3.5	59.9	60.9	1.0
P5a	6.1	58.4	60.2	1.8
P4b	7.8	49.9	52.3	2.4
P5b	12.5	43.4	47.7	4.3

The LCSTs of the polymer solutions exhibited a strong dependence upon the content of incorporated azobenzene to the chain length of the polymer. For polymers with increasing amount of hydrophobic azobenzene chromophore, the LCST decreased and a broadening of the curves was observed. While the LCSTs of **P4a** and **P5a** differed in 1.5 °C, the LCSTs of **P4b** and **P5b** showed a difference of 6.5 °C. Additionally, the polymers **P4b** and **P5b** had lower cloud points than the polymers **P4a** and **P5a**, respectively. This showed that the end group functionality exhibited a stronger influence on the LCST in the case of polymers with a lower molecular weight. The LCST values of the polymers **P4a**, **P5a**, **P4b** and **P5b** were plotted in versus the amount of azobenzene (see figure 6). However, the polymers **P1a** and **P1b** had the lowest LCST values within a polymer series with the same molecular weight due to the hydrophobic effect of the dithioester and PFP-ester end groups.

The content of azobenzene had been calculated by several methods combining ^{1}H NMR spectroscopy and UV/Vis measurements. Therefore a total error of 10 % was assumed for each calculated amount of azobenzene end group, while the errors for the LCST values measured were in a range of ± 0.3 °C.

As the polymers contained photoswitchable azobenzene either on one side (**P4a**, **P4b**) or on both sides of the chain end (**P5a**, **P5b**), a light controlled solubility change was investigated. The isomerization of azobenzene is always accompanied with a change in dipole moment of the molecular structure. For the synthesized polymers containing azobenzene groups, this means that the LCST can be shifted through irradiation with UV-light. For that, the respective samples were irradiated with UV-light of 365 nm for 1 hour and the cloud point of the solutions was measured again. In all cases, higher LCST-values were observed after irradiation (see figure 6).

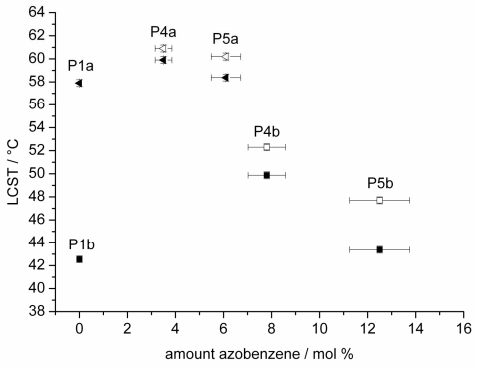


Figure 6. *Dependency of the LCST values of the two P(OEGMA) polymer series a (triangle) and b (rectangle) on the amount of azobenzene end groups. The polymers P4a, P5a, P4b and P5b showed higher LCST values after irradiation (white dots) than before irradiation (black dots) with UV-light.*

As an example, the LCST curves of the polymers **P4b** and **P5b** before and after irradiation are illustrated in figure 7. The LCST shift can be explained by the isomerisation of the azobenzene groups accompanied with an increase in dipole moment and thus an increased local polarity present at the polymer backbone. Accordingly, within this temperature range an isothermal, light- induced precipitation of the copolymers was possible. The values for the LCST of the irradiated solutions and

the LCST shifts are listed in table 3. Noteworthy, the LCST shifts between irradiated and non-

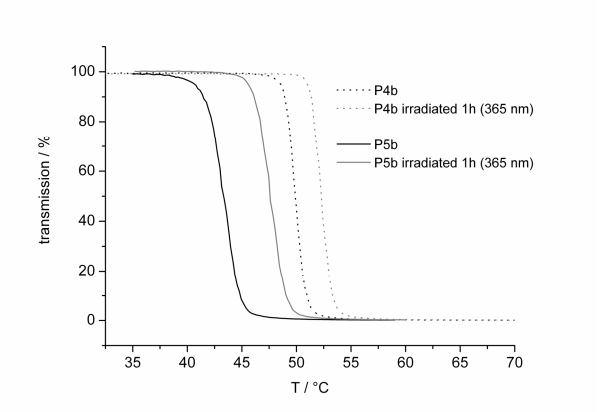


Figure 7. *LCST heating curves of the polymers P4b (dotted curves) and P5b (line curves) before (black) and after irradiation with UV-light of 365 nm for 1 h (grey). The concentration was 10 mg/mL in Millipore water and the heating rate 1°C/min.*

irradiated solution increased linearly with increasing amount of azobenzene chromophore (see figure 8), which is in strong contrast to the transition behavior of copolymers based on poly(*N*-alkylacrylamides) with azobenzene sidegroups.[18,22]

Table 3. *Composition and LCST values for the P(OEGMA) polymers.*

P.	amount of azo-benzene [mol %] *(measured by UV/Vis)*	LCST before irradiation [°C]	LCST after irradiation [°C]	Δ LCST [°C]
P4a	3.5	59.9	60.9	1.0
P5a	6.1	58.4	60.2	1.8
P4b	7.8	49.9	52.3	2.4
P5b	12.5	43.4	47.7	4.3

This finding is, however, in accordance to a similar linear LCST increase which was already reported by Akiyama et al. for PNIPAM based thermoresponsive polymer with one azobenzene end group.[26] In general we observed the same effect for P(OEGMA) polymers with either one or two terminal azobenzene end group functionality. Noteworthy, the polymer **P5a** with azobenzene at both end groups showed an almost twice higher LCST shift (1.8°C) than the polymers **P4a** with one azobenzene terminus (1.0°C). The same effect was observed for the polymer pairs **P5b** (4.3°C) and

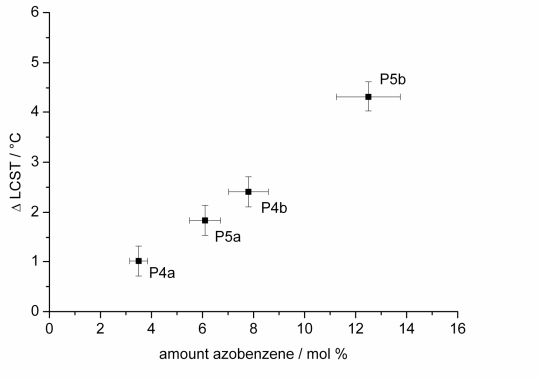


Figure 8. *LCST shifts of the P(OEGMA) polymers versus the amount of azobenzene end groups. The differences in the LCST between the irradiated and none irradiated solutions increased linearly with increasing amount of azobenzene end groups.*

P4b (2.4°C). We believe that the presence of azobenzene at the terminus of each polymers result in the controlled LCST shifts, as the photoisomerization is totally independent from side group effects which might occur in copolymers. To prove the reversibility of the light induced solubility change, the samples were allowed to stand for several hours at daylight. LCST measurements were performed again and showed the same values for the cloud points as measured before irradiation with UV-light. Additionally, UV/Vis kinetics of the irradiated solutions were measured (see supporting information). While the light (daylight) induced reisomerization of the azobenzene occurred very fast ($\tau_{1/2}$ = 5 min), the thermal (room temperature) reisomerization in the dark had a half time of 14 days.

Conclusion

In summary, we have presented the synthesis of P(OEGMA) polymers with either α– or telechelic azobenzene functionalities exhibiting a thermo- and light responsive behavior in aqueous solution. Within the investigated polymers, the molecular weight distribution was narrow, which could be

achieved by RAFT polymerization using a PFP-functionalized chain transfer agent (CTA) yielding polymers with defined molecular weight and one activated end-group per chain. The two polymers synthesized had different molecular weights (**P1a**: M_n(NMR) = 7900 g mol^{-1}, **P1b**: M_n(NMR) = 3800 g mol^{-1}) and consequently a different ratio between the repeating units to the end groups. By using the PFP-functionalized azo compound we were able to substitute the dithioester endgroup of the polymers into a PFP-ester thus allowing active ester functionalization at the ω-end of the polymer chain. Both the α and the ω PFP-ester end groups could be addressed with primary amino-functionalized azobenzene with high conversion yielding in light- and temperature responsive polymers. The obtained polymers **P4a-b** and **P5a-b** exhibited a LCST in aqueous solution that depended strongly on the amount of incorporated azobenzene as well as the ratio between azobenzene endgroup and the molecular weight of the polymer. Furthermore, the reversible isomerization of the azobenzene end groups in the polymers, which was induced by irradiation with UV-light, had an influence on the LCST. Higher LCST values were measured after irradiation and thus, in the temperature region between the LCST of the non-irradiated and the irradiated solution, a light controlled reversible solubility change was found. A maximum LCST shift of 4.3°C was measured for the polymer **P5b**. Additionally, a linear increase in the LCST shifts with an increasing amount of azobenzene in the P(OEGMA) polymers was observed, which was in contrast to the phase transition behavior of thermo responsive polymers with azobenzene side groups.

Acknowledgement

F. D. Jochum gratefully acknowledges the University of Mainz for financial support. P. J. Roth gratefully acknowledges support of the International Research Training Group (IRTG 1404) funded by the DFG. J. Podszuweit is acknowledged for support in the experimental work.

References

(1) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173-1222.

(2) Galaev, I. Y.; Mattiasson, B. *Trends Biotech.* **1999**, *17*, 335-340.

(3) Hoffman; A. S.; Stayton, P. *Macromol. Symp.* **2004**, *207*, 139-151.

(4) Lutz, J.-F. *Polym. Int.* **2006**, *55*, 979-993.

(5) Plate, N. A.; Lebedeva, T. L.; Valuev, L. I. *Polymer Journal* **1999**, *31*, 21-27.

(6) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2001**, *34*, 1391-1399.

(7) Idziak, I.; Avoce, D.; Lessard, D. *Macromolecules* **1999**, *32*, 1260-1263.

(8) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163-249.

(9) Lutz, J.-F.; Akdemir, Ö.; Hoth, A. *J. Am. Chem. Soc.* **2006**, *128*, 13046-13047.

(10) Lutz, J.-F.; Hoth, A. *Macromolecules* **2006**, *39*, 893-896.

(11) Lutz, J.-F. *J. Polym. Sci. A. Polym. Chem.* **2008**, *46*, 3459-3470.

(12) Jones, J. A.; Novo, N.; Flagler, K.; Pagnucco, C. D.; Carew, S.; Cheong, C.; Kong, X. Z.; Burke, N. A. D.; Stöver, H. D. H. *J. Polym. Sci. A. Polym. Chem.* **2005**, *43*, 6095-6104.

(13) Li, D.; He, Q.; Yang, Y.; Moehwald, H.; Li, J. *Macromolecules* **2008**, *41*, 7254-7256.

(14) Magnusson, J. P.; Khan, A.; Pasparakis, G.; Saeed, A.; Wang, W.; Alexander, C. *J. Am. Chem. Soc.* **2008**, *130*, 10852-10853.

(15) Wang, G.; Tong, X; Zhao, Y. *Macromolecules* **2004**, *37*, 8911-8917.

(16) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(17) Kroeger, R.; Menzel, H.; Hallensleben, M. L. *Macromol. Chem. Phys.* **1994**, *195*, 2291-2298.

(18) Akiyama, H.; Tamaoki, N. *J. Polym. Sci. A. Polym. Chem.* **2004**, *42*, 5200-5214.

(19) Kungwatchakun, D.; Irie, M. *Makromol. Chem. Rapid. Commun.* **1988**, *9*, 243-246.

(20) Shimoboji, T.; Larenas, E.; Fowler, T.; Hoffman, A. S.; Stayton, P. S. *PNAS* **2002**, *99*, 16592-16596.

(21) Luo, C.; Zuo, F.; Ding, X.; Zheng, Z.; Cheng, X.; Peng, Y. *J. of Appl. Polym. Sci.* **2008**, *107*, 2118-2125.

(22) Jochum, F. D.; Theato, P. *Polymer* **2009**, *50*, 3079-3085.

(23) Hartley, G. S. *Nature* **1937**, *140*, 281-282.

(24) Yager, K. G.; Barrett, C. J. *Journal of Photochemistry and Photobiology A: Chemistry* **2006**, *182*, 250-261.

(25) Hartley, G. S.; LeFevre, R. J. W. *J. Chem. Soc. 1939***,** 531-534.

(26) Akiyama, H.; Tamaoki, N. *Macromolecules* **2007**, *40*, 5129-5132.

(27) Kopping, J. T.; Tolstyka, Z. P.; Maynard, H. D. *Macromolecules* **2007**, *40*, 8593-8599.

(28) Lutz, J.-F.; Börner, H. G.; Weichenhan, K. *Macromolecules* **2006**, *39*, 6376-6383.

(29) Opsteen, J. A.; van Hest, J. C. M. *Chem. Commun.* **2005**, 57-59.

(30) Matyjaszewski, K.; Nakagawa, Y.; Gaynor, S. G. *Macromol. Rapid Commun.* **1997,** *18*, 1057-1064.

(31) Postma, A.; Davis, T. P.; Moad, G.; O'Shea, M. S. *React. Funct. Polym.* **2006**, *66*, 137-147.

(32) Deng, G.; Chen, Y. *Macromolecules* **2004**, *37*, 18-26.

(33) Mantovani, G.; Lecolley, F.; Tao, L.; Haddleton, D. M.; Clerx, J.; Cornelissen, J. J. L. M.; Velonia, K. *J. Am. Chem. Soc.* **2005**, *127*, 2966-2973.

(34) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754-6756.

(35) Qiu, X.-P.; Winnik, F. M. *Macromol. Rapid. Commun.* **2006**, *27*, 1648-1653.

(36) Postma, A.; Davis, T. P.; Evans, R. A.; Li, G.; Moad, G.; O'Shea, M. S. *Macromolecules* **2006**, *39*, 5293-5306.

(37) Perrier, S.; Takolpuckdee, P.; Mars, C. A. *Macromolecules* **2005**, *38*, 2033-2036.

(38) Chen, M.; Ghiggino, K. P.; Mau, A. W. H.; Rizzardo, E.; Thang, S. H.; Wilson, G. J. *Chem. Commun.* **2002**, 2276-2277.

(39) Moad, G.; Chong, Y. K.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polymer* **2005**, *46*, 8458-8468.

(40) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559-5562.

(41) Roth, P. J.; Kessler, D.; Zentel, R.; Theato, P. *J. Polym. Sci. A. Polym. Chem.* **2009**, *47*, 3118-3130.

(42) Roth, P. J.; Wiss, K. T.; Zentel, R.; Theato, P. *Macromolecules* **2008**, *41*, 8513-8519.

(43) Wiss, K. T.; Krishna, O. D.; Roth, P. J.; Kiick, K. L.; Theato, P. *Macromolecules* **2009,** *42*, 3860-3863.

(44) Roth, P. J.; Kim, K.-S.; Bae, S. H.; Sohn, B.-H.; Theato, P.; Zentel, R. *Macromol. Rapid. Commun.* **2009**, *30*, 1274-1278.

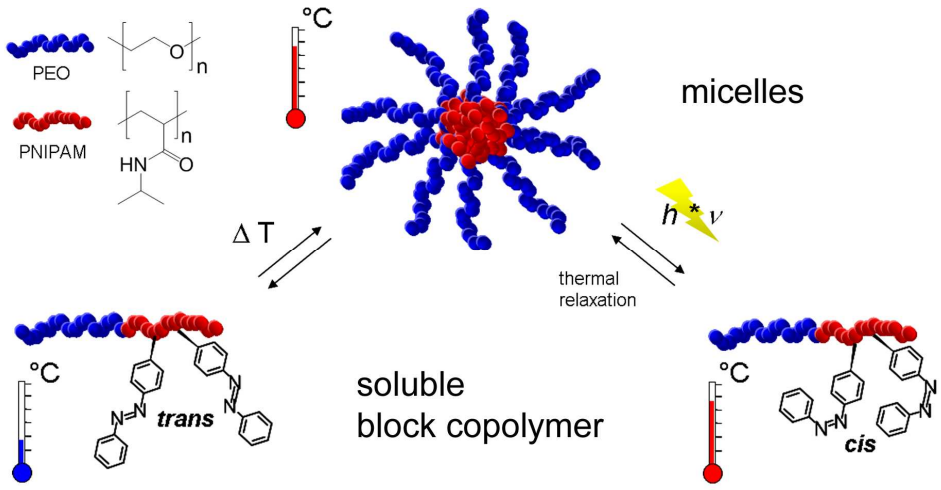
(45) Thang, S. H.; Chong, Y. K.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E. *Tetrahedron Lett.* **1999**, *40*, 2435-2438.

Thermo- and Light Responsive Micellation of Azobenzene Containing Block Copolymers

*Florian D. Jochum, Patrick Theato**

ABSTRACT

In this communication, the synthesis and characterization of thermo- and light responsive block copolymers is reported. PEO-*b*-PNIPAM polymers with azobenzene moieties were prepared and analyzed by turbidimetry, fluorescence, NMR and DLS measurements. A temperature controlled reversible formation as well as a light induced disruption and reformation of micellar structures in water was found.



Introduction

Stimuli-responsive polymers show sharp responses to environmental changes such as pH, temperature, redox or chemical reactions.[1,2] Amphiphilic block copolymers are prone to form polymeric micelles upon self-assembly in aqueous solution. The resulting micellar nanostructures have been proposed for several nanotechnological applications. Besides the aforementioned stimuli, also light as a new stimuli has attracted great attention.[2-9] Recently, photoreversible morphological changes of block copolymer micelles have been reported.[8,9] Besides, the temperature controlled aggregation of block copolymers based on the poly(ethyleneoxide)-*block*-poly(*N*-isopropyl-acrylamide) (PEO-*b*-PNIPAM) has been investigated.[10] This block copolymer showed the interesting property of a reversible temperature responsive formation of micelles above the lower critical solution temperature (LCST) of the PNIPAM block.[10] However, only few studies that report on light and temperature controlled micellar assembly of block copolymers with chromophores in the PNIPAM block exist up-to-date[11,12]

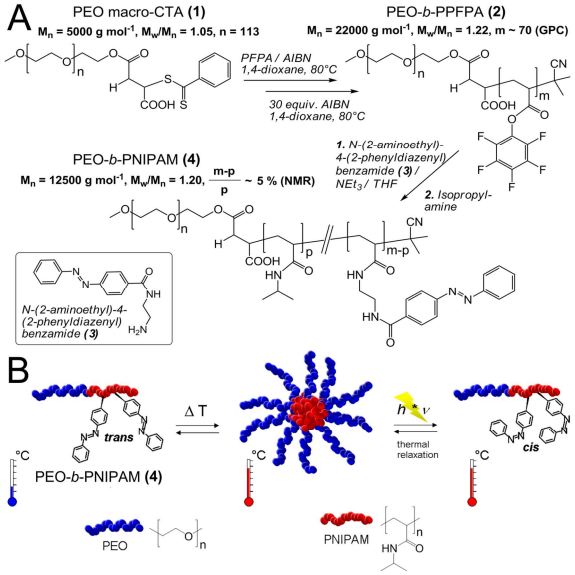
In this communication, we report on the synthesis and characterization of thermo- and light responsive amphiphilic block copolymers and their reversible formation and disruption of polymeric micelles. Motivated by earlier work, we developed a new synthetic procedure toward PEO-*b*-PNIPAM block copolymers containing phototoswitchable azobenzene moieties in the thermo responsive PNIPAM block. Azobenzene is known to undergo *cis-trans* isomerization on alternating irradiation with UV and visible light.[4] Noteworthy, this isomerization is accompanied by a change in the dipole moment of the chromophore and thus the polarity.[4] We recently demonstrated that the LCST of PNIPAM homopolymers containing light responsive groups depends on the isomerization state of the photochromic dye.[4,5]

Experimental Section

PEO-*b*-PNIPAM (**4**) block copolymers containing azobenzenze side groups in the PNIPAM block were synthesized via a polymeranalogous reaction of poly(ethyleneoxide)-*block*-poly(pentafluoro-phenylacrylate) (PEO-*b*-PPFPA, **2**), which was synthesized by RAFT polymerization, as shown in scheme 1. The dithioester end group of PEO-*b*-PPFPA (**2**) was removed by treatment with an excess amount of AIBN. The removal of the dithioester was crucial to prevent the formation of disulfide polymer-polymer dimers during aminolysis. The final PEO-*b*-PPFPA (**2**) had a molecular weight M_n = 22.000 g mol^{-1} (determined by GPC) corresponding to an average number of appraximately 70 activated ester repeating units, with a molecular weight distribution of M_w/M_n = 1.22.† Additionally, ^{1}H NMR and FT-IR measurements proved a successful formation of the block copolymer.†

PEO-*b*-PPFPA (**2**) was allowed to react. with *N*-(2-aminoethyl)-4-(2-phenyldiazenyl)-benz-amide (**3**), followed by reaction with an excess of isopropylamine in THF at 50°C. The resulting PEO-*b*-PNIPAM (**4**) block copolymer with an average amount of 5 mol% azobenzene side groups in the thermoresponsive PNIPAM block was purified by dialysis against water and several precipitation steps. Again, a successful reaction was demonstrated by ^{1}H NMR and FT-IR measurements.† The GPC curve was shifted to lower elution volume as the molecular weight of the PEO-*b*-PNIPAM (**4**) was M_n = 12.500 g mol^{-1} (M_w/M_n = 1.20), which was lower when compared to the molecular weight of the precursor polymer PEO-*b*-PPFPA (**2**).† The

amount of incorporated azobenzene was calculated by ^{1}H NMR integration of the proton signals of the azobenzene aromatic protons (8.09-7.52 ppm) and the PNIPAM singlet (3.96 ppm).† UV/Vis measurements in water showed the characteristic absorption bands (326 nm and 431 nm) of the azobenzene side groups.



Scheme 1. *A. Synthesis of azobenzene containing PEO-b-PNIPAM (4) combining RAFT polymerization and polymer analogous reactions; B. Schematic temperature induced micellation and light controlled micelle disruption/thermal reformation of PEO-b-PNIPAM (4) in water.*

Results and Discussion

The self-assembling behavior of PEO-*b*-PNIPAM (**4**) into micellar structures in response to external stimuli, i.e. temperature and light (see scheme 1) was investigated by several complementary methods. For these systems, the critical micelle temperature (CMT) is crucial and is usually in the range of the LCST of PNIPAM. Above this temperature, the PNIPAM block is insoluble in water and forms the hydrophobic core of a micellar structure, while the PEO block forms the hydrophilic corona.

The CMT, was determined for PEO-*b*-PNIPAM (**4**) by turbidimetry. A concentrated solution (10 mg/mL in H_2O) of the block copolymer was heated in a sample cell of an UV/Vis spectrometer and the transmission of the light beam (λ = 632 nm) was recorded against the temperature (see figure 1). Clearly, the transmittance decreased upon heating in the range from 20°C to 50°C. However, as can be seen, the transmittance did not decrease completely and the solution at 50°C was macroscopic still transparent and not turbid. This well-known phenomena is explained by the formation of micellar structures, which scatter in the case of higher temperatures and thus reduce the transmission of a light beam.[13]

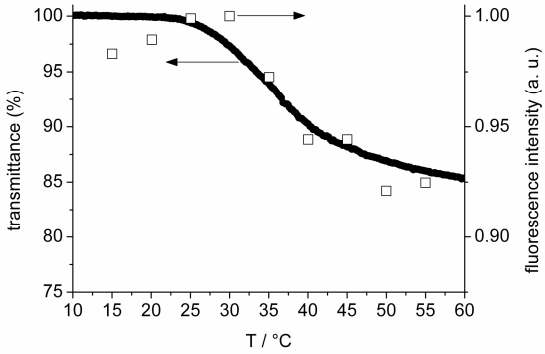


Figure 1. *Turbidimetry measurements of PEO-b-PNIPAM (4, black curve) and temperature dependent fluorescence measurements (rectangle) of a solution of PEO-b-PNIPAM (4) in presence of coumarin 102.*

Next, temperature dependent ^{1}H NMR spectroscopy was performed. A solution (7 mg/mL) of the PEO-*b*-PNIPAM (**4**) in D_2O was measured at 20°C, 40°C and 60°C, respectively. The ^{1}H NMR spectra showed a reduced intensity and broadening of the signals of the PNIPAM block at 40°C and 60°C when compared to the signals at 20°C.†

Additionally, temperature dependent fluorescence spectroscopy was applied to investigate the reversible formation of micellar core-shell structures upon heating PEO-*b*-PNIPAM (**4**) solutions. We attempted to utilize the micelle formation, for reversible capture and release of hydrophobic dyes. For that, 5 mg of **4** was dissolved in 10 mL of a concentrated solution of coumarin 102 in water, to guarantee a

full encapsulation of the dye. The fluorescence was measured at different temperatures from 15°C to 55°C in steps of 5°C (see figure 1). The initial solution showed the strong emission of the coumarin dye at 485 nm. The intensities decreased when the solution was heated from 30°C to 50°C, as a result of the quenching effects once several dye molecules are in close proximity inside the micelle core. Although, the fluorescence intensities did not decrease completely, due to the excess amount of dye, this measurement confirmed the encapsulation of hydrophobic dye in the core of the formed micelles. This experiment was fully reversible as the initial fluorescence intensities were obtained when cooling back to 15°C. The fluorescence intensities maxima at 485 nm were plotted versus the temperature, as shown in figure 1 and indicated a similar trend as the turbidimetry measurements.

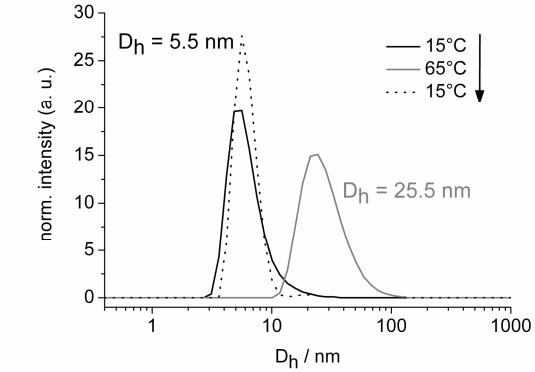


Figure 2. *DLS measurements of PEO-b-PNIPAM (**4**) at different temperatures showing the reversible formation of micellar structures.*

Further, dynamic light scattering (DLS) measurements were conducted to investigate the change of the hydrodynamic diameter D_h of the block copolymer below and above the CMT. Therefore, an aqueous polymer solution (1 mg/mL) was measured at two different temperatures: 15°C and 65°C. At 15°C, the block copolymer was molecularly dissolved and the hydrodynamic diameter was measured to be D_h = 5.5 nm (see figure 2).

After increasing the temperature to 65°C, the hydrodynamic diameter increased to an average value of D_h = 25.5 nm, indicating the formation of micelles as the measured value corresponds to typical sizes of polymeric micelles.[1] After cooling to 15 °C the size distribution decreased and was the found to be the same as prior heating.

The light induced isomerization of azobenzene dyes from *trans* to *cis* is accompanied by a change of the local polarity. As the transition between a molecular dissolved block copolymer and a micellar structure did not occur at a fixed temperature, such as the LCST, it is consequently a challenge to measure a light responsive behavior of the block polymer for all polymer chains simultaneously. At a fixed temperature above the CMT, the micelles should disrupt after irradiation due to the increased polarity of the PNIPAM with azobenzene side groups in the *cis*-configuration (see scheme 1).

The light induced reversible isomerization of **4** was investigated through UV/Vis measurements. Therefore, the isomerization of the azobenzene side groups followed by either thermal or visible reisomerization was analyzed. As a result, the kinetics followed the same process as earlier reported for PNIPAM copolymers with azobenzene side groups.[4]

The light responsive micellar disruption was demonstrated by turbidimetry and DLS. As already mentioned, changes of the transmittance can be used to investigate a micellation process, because micelles are larger scattering objects and thus cause a decrease of the tranmittance.[13] An aqueous polymer solution (10 mg/mL) of the PEO-*b*-PNIPAM (**4**) was heated to a constant temperature of 30°C, i. e. slightly above the CMT, inside the UV/Vis spectrometer with a setup reported earlier.[3]

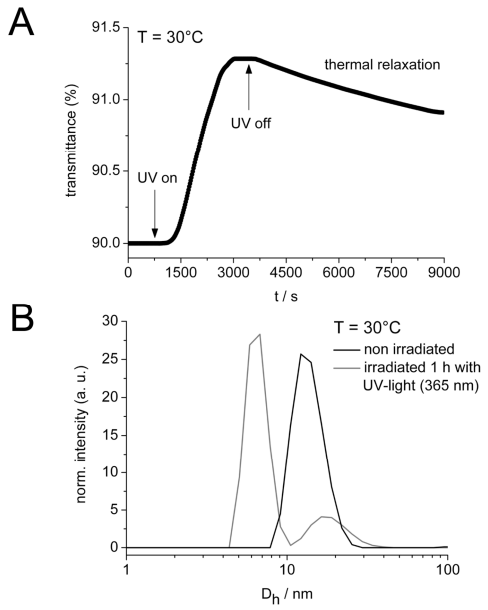


Figure 3. *A. Turbidimetry kinetics of 4 at 30°C showing a partially light induced (λ = 365 nm) disruption of micelles followed by a thermal reformation; B. DLS measurements at 30°C of 4 before and after irradiation with UV-light (1 h, λ = 365 nm).*

At 30°C, most of the PEO-*b*-PNIPAM (**4**) should be self-assembled into polymeric micelles and a kinetic measurement of the transmittance of the light beam (λ = 632 nm) was performed, by plotting the intensity of the light transmitting the quartz cuvette at 30°C versus time (see figure 3). The transmittance remained constant at constant temperature (see first 750 s). After 750 s the sample solution was irradiated with UV-light of λ = 365 nm using an optical fiber attached to a 500W UV-lamp (6.5 mW/cm^2) from above.[3] The transmittance increased notably (1.4 %) once irradiation has been started and reached an equilibrium after 38 min of irradiation (3000 s). After 3400 s the UV-light was switched off. As a consequence, thermal reisomerization of the azobenzene started thus decreasing the transmittance slowly ($\tau_{1/2}$ ~ 6 h).

Further, DLS measurements of a irradited solution of **4** (1 mg/mL) at 30°C, i. e. the temperature were the micellation process starts, showed smaller structures. The average size distributions was smaller toward a non-irradited solution (see figure 3).

Although this effect may only occur in a small temperature range, these experiments clearly demonstrated for the first time the successful light disruption and reformation of a micellar structure with a chromophore attached inside the core at a constant temperature.

Conclusion

In summary, we synthesized and characterized stimuli- responsive polymers in respect to their self-assembly into micellar structures. The synthesis was based on a polymer analogous reaction of PEO-*b*-PPFPA (**2**) precursor polymers with amines.

The resulting block copolymer PEO-*b*-PNIPAM (**4**) formed temperature dependent micellar structures in water. The reversible formation was thoroughly investigated by temperature dependent NMR and fluorescence measurements, as well as turbidimetry and DLS. Light controlled disruption and thermal reformation of the micelles, was successfully demonstrated by transmittance and DLS measurements.

Acknowledgement

Support of the work by the University of Mainz and the WCU (World Class University) program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (400-2008-0230) are acknowledged. S. Bonenberger and F. R. Forst are gratefully acknowledged for experimental support. M. Helm and M. Hirsch (Institute of Pharmacy) are acknowledged for fluorescence measurements. The Institute of Biophysics is acknowledged for DLS measurements.

References

(1) Lazzari, M.; Liu, G.; Lecommandoux, S. *Block Copolymers in Nanoscience* **2006**, Wiley-VCH.

(2) Jiang, J.; Tong, X.; Morris, D.; Zhao, Y. *Macromolecules* **2006**, *39*, 4633-4640.

(3) Jochum, F. D.; Theato, P. *Macromolecules* **2009**, *42*, 5941-5945.

(4) Jochum, F. D.; zur Borg, L.; Roth, P. J.; Theato, P. *Macromolecules* **2009**, *42*, 7854-7862.

(5) Zhao, Y. *J. Mater. Chem.* **2009**, *19*, 4887-4895.

(6) Lee, H.; Wu, W.; Oh, J. K.; Mueller, L.; Sherwood, G.; Peteanu, L.; Kowalewski, T.; Matyjaszewski, K. *Angew. Chem. Int. Ed.* **2007**, *46*, 2453-2457.

(7) Dai, S.; Ravi, P.; Tam, K. C. *Soft Matter* **2009**, *5*, 2513-2533.

(8) Yan, J.; Ji, W.; Chen, E.; Li, Z.; Liang, D. *Macromolecules* **2008**, *41*, 4908-4913.

(9) Jiang, X.; Lavender, C. A.; Woodcock, J. W.; Zhao, B. *Macromolecules* **2008**, *41*, 2632-2643.

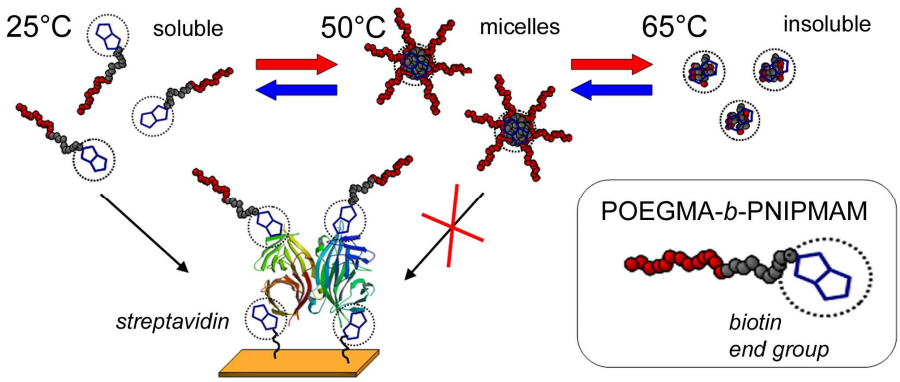
(10) Zhou, Y.; Jiang, K.; Song, Q., Liu, S. *Langmuir* **2007**, *23*, 13076-13084.

Double Thermo-Responsive Block Copolymers Featuring a Biotin End Group

*Florian D. Jochum, Peter J. Roth, Daniel Kessler, Patrick Theato**

ABSTRACT

A poly[(oligo(ethylene glycol) monomethyl ether methacrylate)-*block*-(*N*-isopropyl methacrylamide)] (POEGMA-*b*-PNIPMAM) block copolymer with a biotin end group on the PNIPMAM block as a biotarget was synthesized as a model system for temperature controlled polymer immobilization. The synthesis was based on RAFT polymerization followed by post-modification of an activated ester precursor block and an exchange of the dithioester end group within one step. NMR, differential scanning calorimetry (DSC), dynamic light scattering (DLS) and turbidimetry measurements were performed to investigate the stimulus-responsive properties. The double thermoresponsive POEGMA-*b*-PNIPMAM with biotin end group showed a temperature dependent multi-stage assembly behavior as it was completely soluble in water at temperatures below the LCST of both blocks, formed micellar structures above the LCST of PNIPMAM but below the LCST of POEGMA, or precipitated from solution above the LCST of both blocks. At room temperature, the polymer could be immobilized onto a streptavidin surface via its biotin end group, as shown in surface plasmon resonance (SPR) experiments. At 50°C, at which the block copolymer formed micelles trapping the biotin target within the PNIPMAM core, no immobilization was observed, showing that the biological binding ability of the model could be controlled via external stimuli.



Introduction

Amphiphilic block copolymers are unique materials due to their self-organization behavior in aqueous solution that enables the formation of polymeric micelles.[1] The driving force of the micellation process is generally attributed to the microphase separation of one insoluble block and one soluble block in water.[2,3] Following this principle, the selective alteration of the solubility of one block has led to the reversible formation of micelles that can be induced by inter-polymer complexation,[4,5] chemical transformation,[6,7] the addition of a selective solvent,[8-11] and stimuli-responsive solubility changes of double hydrophilic block copolymers.[12-21] All these approaches have been successfully employed to fabricate micellar nanoparticles possessing core-shell microstructures.

In recent years it has been shown that especially double hydrophilic block copolymers, which exhibit one thermo-responsive block, can result easily in amphiphilic block copolymers upon increase of temperature. Block copolymers containing a hydrophilic water soluble block, like poly(ethylene oxide), and a temperature sensitive block, such as poly(*N*-isopropylacrylamide) (PNIPAM),[22-26] have attracted great attention in the past few decades due to their potential use in drug delivery.[27] Besides PNIPAM, many other polymers that exhibit a LCST in aqueous solution exist: polyacrylamides,[28-30] polymethacrylamides[31] and polymethacrylates with poly(ethylene glycol) side chains[32]. Especially poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) and poly(*N*-isopropyl methacrylamide) (PNIPMAM), the analogous poly(methacrylamide) to PNIPAM, are of special interest for biomedical applications.[32-47] Depending on their molecular structure, the LCST of POEGMA with an average of 4 ethylene glycol repeating units has been reported to be 64°C[32] while the LCST of PNIPMAM is known to be at 45°C.[31] Consequently, it would be interesting to explore the micellation and/or aggregation process of a block copolymer containing both thermo-responsive polymers with different LCSTs. To the best of our knowledge, there have been only a few reports in which multiple thermo-responsive block copolymers or multiblock copolymers have been synthesized and characterized.[38,48-57]

Further, the end groups of polymers are the focus for many biomedical applications, as they allow for a direct conjugation of biological moieties in a one-to-one manner.[58-63] In addition to methods of covalently connecting synthetic materials and, for example, proteins, biology offers the possibility of strong non-covalent bonds based on the bioaffinity of certain proteins toward specific targets. As such, the strong affinity between streptavidin and biotin represents a prime example.[64-69] Recently, thermoresponsive block copolymers capable of forming micelles with biotin end group have been reported.[58,60,70] However, to the best of our knowledge, the appealing idea to combine biotin end group functionalized polymers with double thermo-responsive block copolymers has not yet been explored yet, mainly due to the fact that the necessary synthetic techniques have only become available very recently. Such double thermo-responsive block copolymers featuring a biotin end group would not only be model compounds for smart micelles that can be used for drug delivery, in which the drug is immobilized by a streptavidin-biotin interaction in the core of a micelle, but also for the preparation of double stimuli-responsive polymer brushes in regard to precisely control cell attachment and cell growth on surfaces.

The scope of the present study is twofold. We present a suitable synthetic route to obtain a double thermo-responsive block copolymer poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(*N*-isopropyl meth-acrylamide) (POEGMA-*b*-PNIPMAM) containing

a functional biotin end group that is able to bind to the protein streptavidin. Therefore, reversible addition-fragmentation chain transfer (RAFT) polymerization was used in combination with the reaction of two orthogonally functional groups, i. e. an activated pentafluorophenyl ester for side chain functionalization of one block, and the dithioester end group that allows the modification utilizing functionalized methane thiosulfonates. Additionally, not only the self-assembling behavior of POEGMA-*b*-PNIPMAM capable of forming polymeric micelles will be investigated, but also the temperature dependent immobilization of these biotin end-group functionalized block copolymers on streptavidin-coated surfaces will be studied.

Experimental Section

Materials. All chemicals and solvents were commercially available and used as received unless otherwise stated. Dry *N*,*N*-dimethylformamide (DMF) was purchased from Sigma-Aldrich. *N*-biotinylaminoethyl methanethiosulfonate (**5**) was purchased from Toronto Research Chemicals Inc. Phosphate buffered saline (PBS) contained 8.0 g/L of NaCl, 0.2 g/L of KCl, 1.44 g/L of Na_2HPO_4, and 0.24 g/L of KH_2PO_4 and was sterilized by boiling. Tetrahydrofuran (THF) and 1,4-dioxane were distilled over sodium. 2,2'-Azobis(isobutyronitrile) (AIBN) was recrystallized from diethyl ether and stored at -7°C. Poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA, **1**) with a molecular weight of M_n(GPC) = 8600 g mol^{-1} was synthesized as reported previously.[71] Pentafluorophenyl methacrylate (PFPMA, **2**) was synthesized as described recently.[72] Streptavidin immobilized surfaces were prepared according to a literature procedure as follows.[73] Briefly, amino-functionalized biotin was synthesized by activation of the carboxyl group with pentafluorophenyl trifluoroacetate, followed by a reaction with an excess of ethylene diamine. The PMSSQ-PPFPA active ester surface coating on top of a gold-covered glass slide was obtained by spin-coating from 0.04 wt% solution of PMSSQ-PPFPA in THF and annealing at 130 °C for 2 h. The surface-analogous reaction of amino-functionalized biotin with the pentafluorophenyl esters at the surface was carried out by dipping the coated substrate into a 10 wt% solution of H_2N-biotin in water for 60 min. After successful attachment of the specific binding site, streptavidin was allowed to adsorb onto the biotinylated coating. For that, the glass slides were placed in a solution of 0.1 g/L streptavidin in phosphate-buffered saline solution (PBS) overnight and washed with buffered solution several times afterwards. Spectra/Por 3 (MWCO 3500) membranes were used for dialysis.

Instrumentation. All ^1H, ^{19}F and ^{13}C NMR spectra were recorded on a Bruker 400 MHz FT-NMR spectrometer in deuterated solvents. Chemical shifts (δ) were given in ppm relative to tetramethylsilane (TMS). Gel permeation chromatography (GPC) was performed in THF to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards. Calibration was done using polystyrene standards. IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer using an ATR unit. Cloud points were determined in Millipore water at a concentration of 25 g/L and were observed by optical transmittance of a light beam (λ = 632 nm) through a 1 cm sample quartz cell. The measurements were performed in a Jasco V-630 photospectrometer with a Jasco ETC-717 peltier element. The intensities of transmitted light were recorded versus temperature of the sample cell. The heating rate was 1°C per minute. Dynamic light scattering (DLS) was measured on a Malvern Zetasizer Nano-S in a low volume glass cuvette (40 μL) on 1 g/L buffered aqueous solutions at an angle of 90°. The solution was filtered through a 0.2 μm PTFE filter prior analysis. Surface plasmon

resonance (SPR) measurements were performed on 1 g/L aqueous buffer solutions (PBS) at room temperature in a self-built cell of 0.5 mL volume using a Θ/2Θ setup, a 632 nm HeNe laser, and a photodiode. Glass slides were coated with 1.5 nm of chromium and 50 nm of gold by evaporation. SPR scans were fitted using WINSPALL[74], assuming a refractive index of all organic materials of 1.5. Differential scanning calorimetry (DSC) measurements were carried out on a Perkin Elmer DSC 7 calibrated by lead and indium standards. Measurements were performed in a temperature range from 10°C to 80°C. The heating/cooling rate was 5°C/min and the concentration of the sample solution in the DSC pan was 25 g/L. The corresponding peak maxima were used for the determination of the LCST by DSC.

Synthesis of poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(pentafluorophenyl methacrylate) (POEGMA-*b*-PPFPMA, 3). 0.702 g (0.0816 mmol) of POEGMA (**1**), 1.028 g (4.079 mmol) of PFPMA (**2**) and 2.3 mg (0.0140 mmol) of AIBN were placed into a Schlenk-tube. After addition of 10 mL of dry 1,4-dioxane, four freeze-pump-thaw cycles were performed to degas the solution. The flask was filled with argon, immersed into a preheated oil bath of 80°C and stirred overnight. After cooling down to room temperature, the block copolymer was isolated by precipitation into hexane. The crude product was dissolved in dry THF, precipitated again three times into hexane, centrifuged and finally dried in vacuum. 830 mg (48 %) of waxy, pink colored POEGMA-*b*-PPFPMA (**3)** were obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 4.09 (br s), 3.83 (br s), 3.65 (br m), 3.56 (br t), 3.38 (s), 2.88 (br s), 2.40 (br s), 2.10 (br s) 1.83 (br d), 1.40 (br d), 0.95 (br d); ^{13}C NMR ($CDCl_3$): δ /ppm: 177.3, 172.7, 142.3, 139.9, 139.2, 136.7, 124.6, 71.9, 70.5, 68.5, 63.8, 59.0, 54.2, 46.0, 44.8, 18.5, 16.8; ^{19}F NMR ($CDCl_3$): δ /ppm: -162.0 (br s, 2F), -156.9 (br s, 1F), -151.4 (br m, 1F), -150.4 (br d, 1F); FT-IR (ATR-mode): 2875 cm^{-1} (C-H valence band), 1780 cm^{-1} (C=O reactive ester band), 1728 cm^{-1} (C=O ester band), 1519 cm^{-1} (PFP C=C aromatic band), 1106 (C-O ether band); M_n(GPC) = 18800 g mol^{-1}, M_w(GPC) = 23400 g mol^{-1}, M_w/M_n = 1.24.

Synthesis of poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(*N*-isopropyl methacrylamide) (POEGMA-*b*-PNIPMAM, 4) with biotin end group. 150 mg POEGMA-*b*-PPFPMA (**3**) was dissolved with 50 mg (0.131 mmol) of *N*-biotinylaminoethyl methanethiosulfonate (**5**) in 2 mL of dry DMF. Afterwards, 43.8 µL (0.314 mmol) of triethylamine (TEA) and 54.2 µL (0.633 mmol) of isopropylamine were added, the flask was sealed and stirred overnight under nitrogen atmosphere. Next, the solution was transferred into a dialysis membrane and dialyzed against methanol for 2 days. After evaporation of the solvent, the residue was redissolved in chloroform and precipitated two times into hexane. The product was centrifuged and dried in vacuum at 40°C. 95 mg of waxy colorless POEGMA-*b*-PNIPMAM with a biotin end group was obtained. ^{1}H NMR ($CDCl_3$): δ /ppm: 5.57 (br s), 4.75 (br s), 4.49 (br s), 4.07 (br s), 3.92 (br s) 3.82 (br s), 3.64 (br m), 3.55 (br t), 3.37 (s); 2.29 (br s), 1.84 (br d), 1.20 (br s), 1.12 (br s), 0.93 (br d); ^{13}C NMR ($CDCl_3$): δ /ppm: 177.2, 71.9, 70.6, 68.5, 63.7, 58.9, 54.3, 44.9, 41.6, 22.0, 18.8, 16.6; FT-IR (ATR-mode): 3407 cm^{-1} (amide N-H-valence), 2875 cm^{-1} (C-H valence band), 1722 cm^{-1} (C=O ester band), 1640 cm^{-1} (C=O amide band I), 1522 cm^{-1} (C=O amide band II), 1096 cm^{-1} (C-O ether band); M_n(GPC) = 11400 g mol^{-1}, M_w(GPC) = 13600 g mol^{-1}, M_w/M_n = 1.19.

Results and Discussion

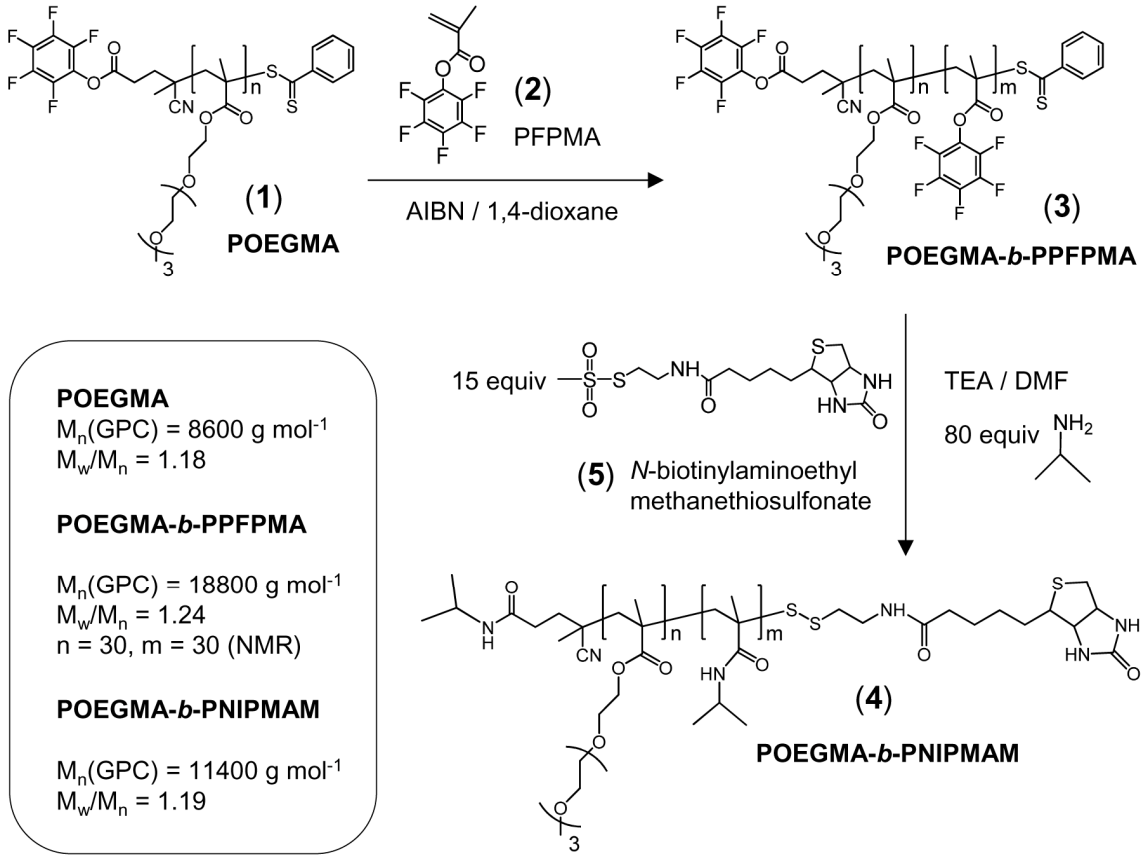
For the preparation of biotin end-group functionalized stimuli responsive block copolymers the technique of RAFT polymerization was

employed. As the first block, oligo(ethylene glycol) methyl ether methacrylate with an M_n of ~ 300 g mol^{-1} was polymerized using pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) as a chain transfer agent, yielding a macro RAFT agent for the following polymerizations.[71,75] The molecular weight of POEGMA (**1**) determined by GPC was M_n = 8600 g mol^{-1}.

Next, poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(pentafluorophenyl methacrylate) (POEGMA-*b*-PPFPMA, **3**) was synthesized by polymerization of pentafluorophenyl methacrylate (PFPMA, **2**) utilizing POEGMA (**1**) as a macro RAFT agent (see scheme 1).

As we could show in previous studies, utilizing activated ester polymers as reactive precursors provides many synthetic advantages for the synthesis of functional polymers.[76-78] In combination with the RAFT polymerization technique, one can obtain precisely defined functional polymer structures, which was successfully demonstrated in manifold examples.[76,79]

Within the present study, successful polymerization of the pentafluorophenyl methacrylate was confirmed by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, FT-IR and GPC measurements. For example, figure 1 (black line) shows the ^{1}H NMR spectrum of POEGMA-*b*-PPFPMA (**3**). The characteristic signals of the backbone of the activated ester block (2.40 ppm and 2.10 ppm) and of the methyl group (1.40 ppm) could be assigned. ^{19}F NMR showed the typical signals at -162.0 ppm, -156.9 ppm -151.4 ppm and -150.4 ppm of the



Scheme 1. *Overview of reactions described in detail in the text.*

PPFPMA polymer block. The four fluorine signals are a consequence of the tacticity of the methacrylate block (see supporting information).

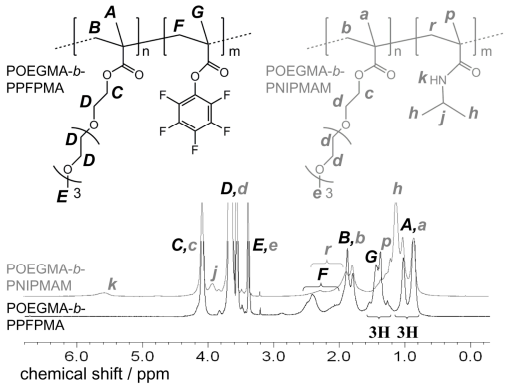


Figure 1. *^{1}H NMR spectra measured in deuterated chloroform of the block copolymers POEGMA-b-PPFPMA (black line) and POEGMA-b-PNIPMAM with biotin end group (gray line).*

Additionally, the FT-IR spectrum (see supporting information) showed in addition to the band for the C=O of the activated ester at 1780 cm^{-1}, the common bands originating from the acrylate ester at 1728 cm^{-1} and the band at 1519 cm^{-1}, which was attributed to the pentafluorophenyl group.

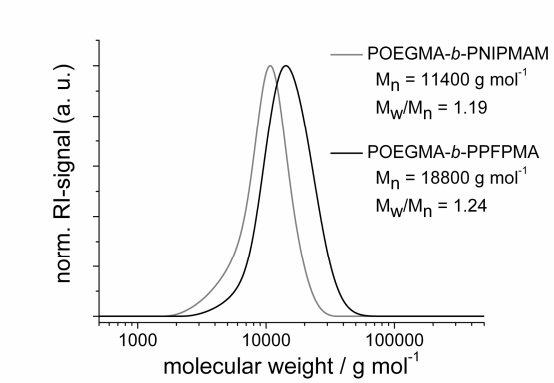


Figure 2. *Normalized GPC elugrams of POEGMA-b-PPFPMA (black line) and POEGMA-b-PNIPMAM with biotin end group (gray line).*

The molecular weight of the block copolymer was determined by GPC. The molecular weight of POEGMA-*b*-PPFPMA (**3**) increased to a value of M_n = 18800 g mol^{-1} with a molecular weight distribution of M_w/M_n = 1.24. The GPC curve of POEGMA-*b*-PPFPMA (**3**) is illustrated in figure 2.

The chain length of the second block was determined through the ratio of the integrals of the methyl groups of the backbone at 1.83 ppm (PPFPMA) and 0.95 ppm (POEGMA) from the proton NMR (see figure 1). A ratio of 1:1 of the two blocks was calculated, which means that both blocks consist of approximately 30 repeating units.

POEGMA-*b*-PPFPMA (**3**) possessed two orthogonally reactive groups, i.e. a block with activated ester repeating units, which are able to react quantitatively with aliphatic amines, and a dithioester end group, originating from the RAFT agent and attached to the PPFPMA block. This approach thus allowed the further modification of the dithioester by means of aminolysis in the presence of a biotinylated methane thiosulfonate (**5**), as shown in scheme 1. The thiol released from the dithioester polymer end group during the aminolysis undergoes a very selective reaction with the electrophilic sulfur of the methane thiosulfonate (MTS) reagent. This method was introduced recently and it allows to quantitatively introduce functional disulfide groups at the end group of polymer chains.[80-82] Disulfides have often been employed as linkers between polymers and biomolecules due to the chemical stability of the S-S-bond as long as reducing agents are avoided.[61,62,68,82-86] The POEGMA-*b*-PPFPMA (**3**) precursor block copolymer was reacted in dry DMF in the presence of triethylamine (TEA) with 80 equiv. of isopropylamine and 15 equiv. of *N*-biotinylaminoethyl methanethiosulfonate (**5**). Consequently, it was possible to convert both the activated ester block and the dithioester end group in a one-pot reaction.

The obtained poly(oligo(ethylene glycol) methyl ether methacrylate)-*block*-poly(*N*-isopropyl methacrylamide) (POEGMA-*b*-PNIPMAM, **4**) possessed two different thermoresponsive blocks and a functional biotin end group. This polymer was purified through dialysis and then analyzed by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, FT-IR as well as GPC measurements.

Figure 1 shows the ^{1}H NMR spectrum of POEGMA-*b*-PNIPMAM (**4**) and of the precursor block copolymer POEGMA-*b*-PPFPMA (**3**). Clearly, the spectra demonstrate the successful reaction of the activated ester side groups with isoproylamine as the peaks of the backbone of the PPFPMA block (2.40 ppm and 2.10 ppm) totally vanished and the characteristic signals of poly(*N*-isopropyl methacrylamide) at 5.57 ppm, 3.92 ppm and 1.13 ppm appeared.

Further, ^{19}F NMR was not able to detect any fluorine signals after the reaction, indicating a complete conversion of all pentafluorophenyl groups (see supporting information). It also showed that all pentafluorophenol salts had been removed during the purification step.

Full conversion of the polymer analogous reaction could additionally be observed by FT-IR spectroscopy (see supporting information) due to the vanishing of the activated ester band at 1780 cm^{-1} and the appearance of the amides bands at 1640 cm^{-1} and 1522 cm^{-1}, respectively.

Again, the molecular weight of the polymer was determined by GPC and a value of M_n = 11400 g mol^{-1} with a molecular weight distribution of M_w/M_n = 1.19 was measured. Consequently, the GPC curve of POEGMA-*b*-PNIPMAM (**4**) was shifted to lower molecular weight, as shown in figure 2. Noteworthy, the molecular weight distribution did not change significantly after the post-modification confirming that no formation of polymer-polymer disulfides had occurred.

The presence of the biotin end group was directly proven utilizing ^{1}H NMR, as a singlet at 4.49 ppm could be assigned to the biotin C-H-protons of POEGMA-*b*-PNIPMAM (**4**) (see supporting information).

Because POEGMA-*b*-PNIPMAM (**4**) with the biotin end group contained different thermo-responsive blocks with different LCSTs, temperature dependent ^{1}H NMR measurements in deuterated water were performed to analyze the phase transition behavior of the block copolymer. Figure 3 shows the ^{1}H NMR spectra of POEGMA-*b*-PNIPMAM (**4**) measured at different temperatures.

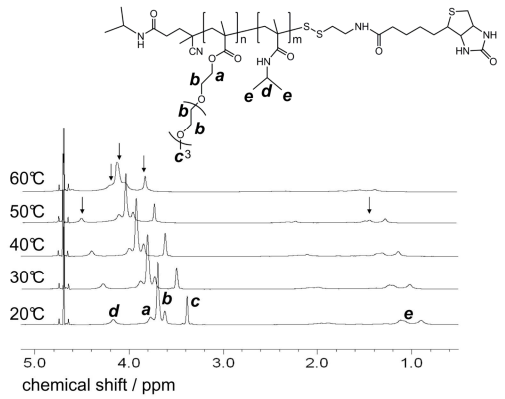


Figure 3. *^{1}H NMR spectra of POEGMA-b-PNIPMAM with biotin end group (10 g/L) in D_2O as a function of temperature, showing the attenuation of the signals corresponding to the two different thermo responsive blocks at higher temperatures.*

The signals at 4.2 ppm and 3.7 ppm are characteristic of the PNIPMAM and POEGMA blocks, respectively (see peak assignment in figure 3). At 20°C, the signals for both blocks are clearly detectable, and all signals are relatively sharp indicating that the diblock copolymer is molecularly dissolved. The relative intensity of the signals that are characteristic for the PNIPMAM block remained unchanged at 30°C and 40°C, respectively. All proton signals were shifted to higher ppm values, but the integral ratio between the POEGMA signal and the PNIPMAM signal was still the same as measured at 20°C. Upon further increasing the temperature to 50°C, the relative intensity of the PNIPMAM signal started to decrease due to the phase separation of the block. On the other hand, the POEGMA signals were still clearly evident, which is reasonable because the POEGMA block remains soluble below 60°C. On the basis of chemical intuition, it is expected that micelles with a PNIPMAM core and a POEGMA corona have formed. At 60°C, the PNIPMAM

signal had almost vanished completely while the signal originating from the POEGMA block started to decrease because the phase transition of the POEGMA block, which was determined beforehand by turbidimetry to be LCST (POEGMA) = 57.9°C, was reached.

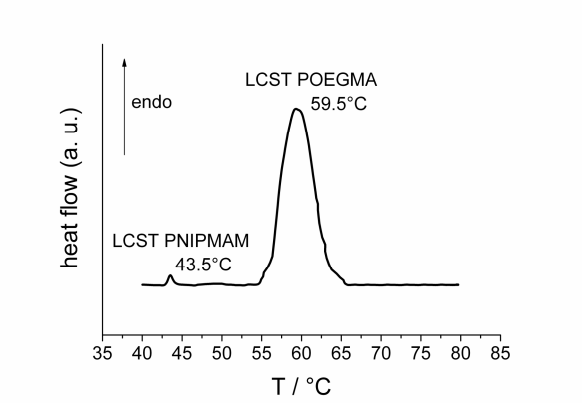


Figure 4. *DSC curve of a 20 g/L aqueous solution of POEGMA-b-PNIPMAM with biotin end group recorded at a heating rate of 5°C/min.*

Further, differential scanning calorimetry (DSC) measurements were performed in order to determine the phase transition temperatures of the block copolymer. Figure 4 shows the heating curve of a concentrated solution (20 g/L) of POEGMA-*b*-PNIPMAM (**4**) in water measured in the range from 10°C to 80°C with a heating rate of 5°C/min. Clearly, the two endothermic peaks corresponding to the different phase transition temperatures of both blocks could be assigned.

For the LCST of PNIPMAM a value of 43.5°C was found, while the POEGMA block had a phase transition temperature of 59.5°C. Both values correspond to phase transition temperatures of the homopolymers respectively, as reported in the literature[31,32]. Noteworthy, the endothermic peak that is assigned to the PNIPMAM block was very small and narrow while the peak of the POEGMA block was very intensive and broad. This phenomenon can be explained by the formation of micelles.

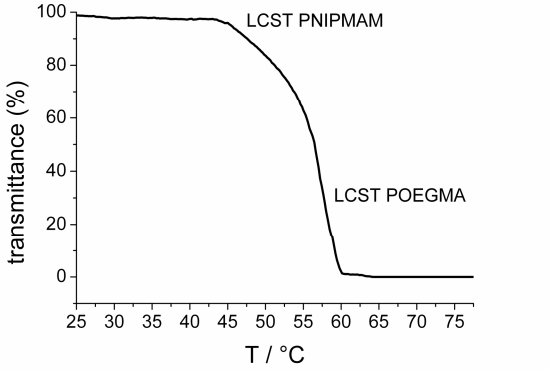


Figure 5. *LCST heating curve of POEGMA-b-PNIPMAM with biotin end group (25 g/L) in aqueous solution.*

Next, cloud point measurements were performed by turbidimetry to analyze the phase transition behavior of the block copolymer. Figure 5 shows the LCST curve measured of POEGMA-*b*-PNIPMAM (**4**). The diagram shows a sharp phase transition beginning at 55°C, which corresponds to the LCST of the POEGMA block. However, a slight decrease in the transmission in the range between 45°C and 55°C was observed. In this region, PNIPMAM undergoes a temperature induced phase transition. This can be explained by the formation of micelles, which reduced the intensity of the transmitted light due to their higher light scattering capability. In general, this measurement confirmed the results obtained by the DSC measurement.

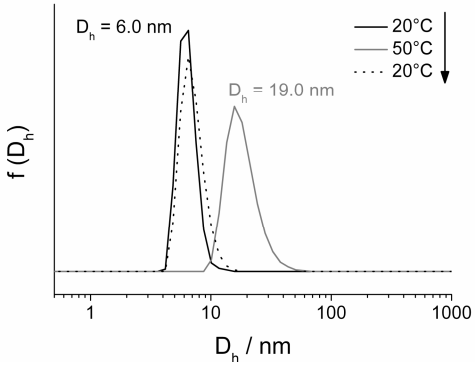


Figure 6. *Temperature dependent change of the hydrodynamic diameter D_h of POEGMA-b-PNIPMAM with biotin end group. A polymer solution (1 g/L) was measured at 25°C and 50°C.*

Additionally, dynamic light scattering (DLS) measurements were performed at different temperatures. Figure 6 shows the evolution of the hydrodynamic diameter of the POEGMA-*b*-PNIPMAM (**4**) as the temperature is changed. At 25°C, both blocks were molecularly dissolved in water and an average value of 6.0 nm was measured for the hydrodynamic diameter D_h of the block copolymer. On increasing the temperature to 50°C, which means above the LCST of the PNIPMAM block but below the LCST of the POEGMA block, a significant change in the size and size distribution could be observed. An average value for the hydrodynamic diameter D_h of 19.0 nm was measured. This value further confirms the plausible formation of micelles due to the temperature-induced collapse of the PNIPMAM block, which forms the hydrophobic micelle core.

The formation of micellar structures was fully reversible, as the hydrodynamic diameter decreased again to the initial value of D_h = 6.0 nm upon cooling to 20°C. These observations were in strong agreement with the conclusion obtained from previous temperature dependant ^{1}H NMR measurement results, which in combination confirm the formation of micelles.

Next, the temperature controlled binding of this POEGMA-*b*-PNIPMAM (**4**) with end capped biotin onto streptavidin surfaces was investigated. As the biotin group is located at the end of the PNIPMAM block, it would be located in the core of the formed micelles when heating a polymer solution of POEGMA-*b*-PNIPMAM (**4**) above the LCST of the PNIPMAM block, i.e. above 43°C. In this case, the biotin end group should not be able to bind onto a streptavidin functionalized surface anymore.

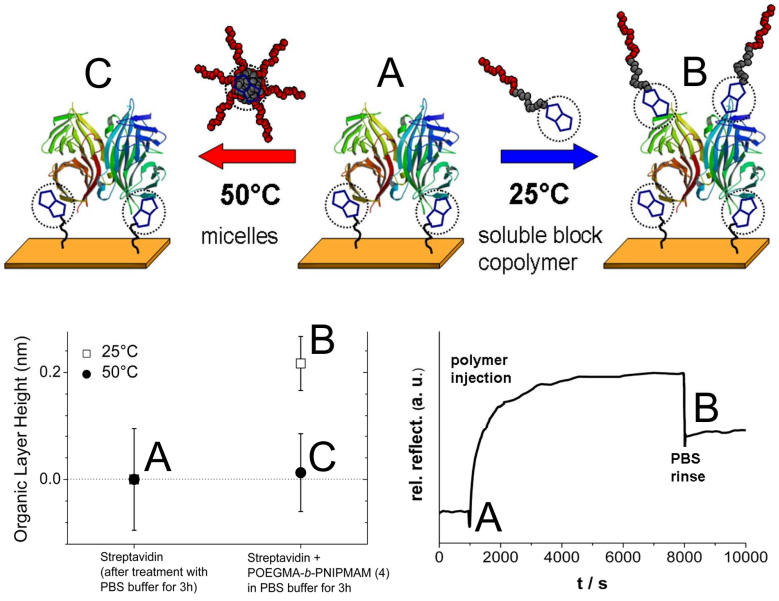


Figure 7. *Surface plasmon resonance (SPR) kinetic measurement of POEGMA-b-PNIPMAM with biotin end group (right graphic). After injection of POEGMA-b-PNIPMAM (1 g/L) in PBS, the reflectance increased due to the physisorption of the block copolymer with its biotin end group onto the streptavidin immobilized surface. The adsorption was completed after 7000 s and the reflectance did not change any longer. In the following, the SPR cell was rinsed several times with PBS to remove any non-specifically bound polymer. The reflectance decreased but remained higher (B) than before the injection (A). This experiment confirmed the presence of the biotin end group.*
Temperature dependence binding of POEGMA-b-PNIPMAM with biotin end group onto a streptavidin immobilized surface (left graphic). The values for the organic layer heights (A, B, C) represent average values of three samples measured by SPR with the calculated standard deviation as error. At 25°C the block copolymer was able to attach onto streptavidin as the organic layer height (0.22 nm) was increased (B) towards the heights before the reaction (A). At 50°C the POEGMA-b-PNIPMAM formed polymeric micelles with the functional biotin end group in the core. As a consequence no adsorption onto the protein surface occurred and the organic layer height (0.01 nm) did not increase significantly (C).

First, to verify the thermal stability of streptavidin immobilized on the surface, three gold covered glass slides with a streptavidin immobilized surface[73] were immersed with a PBS buffer solution at room temperature (25°C) and then in PBS buffer solution at 50°C for 3 hours, respectively. For both series the change of the film thickness was calculated from SPR measurement data using WINSPALL.[74] The average change of the film thickness including the standard deviation error of the three measurements is illustrated in figure 7. Clearly, the thickness of the streptavidin surface did not change after treatment with buffer solution neither at room temperature nor after treatment with warm PBS buffer (50°C). The average increase of the surface was 0.00 nm with a standard deviation of ±0.09 nm. Streptavin is known to be thermally stable up to 65°C.[72] The SPR experiment confirmed that any denaturation of streptavidin by treatment with warm PBS buffer could be excluded.

Next, the polymer with its biotin end group was analyzed in respect to a surface immobilization, which was monitored by surface plasmon resonance (SPR). Therefore, three gold covered glass slides with a streptavidin immobilized layer[73] were placed into a solution (1 g/L) of POEGMA-*b*-PNIPMAM (**4**) in PBS buffer at room temperature (25°C) for 3 hours. A kinetic SPR measurement was performed at room temperature (25°C) and the SPR kinetics of the adsorption of POEGMA-*b*-PNIPMAM (**4**) with the biotin end group onto the streptavidin functionalized surface is shown in figure 7. First, the sample cell was rinsed with phosphate-buffered saline (PBS) solution and the reflectance remained constant (0 to 1000 s). After 1000 s the block copolymer sample in PBS solution was injected and resulted in an increase of the reflectance due to the immobilization of the polymer induced through the selective non-covalent binding of the biotin end group onto the streptavidin surface. The adsorption was completed after 7000 s and the reflectance did not change any longer. In the following, the SPR cell was rinsed several times (8000 s) with PBS solution to remove any non-specifically bound polymer. The reflectance decreased but remained higher than before the injection. A total increase of the film thickness of 0.22 nm was calculated. The selective binding of the block copolymer onto a streptavidin functionalized surface confirmed the presence of the biotin end group.

As summarized in figure 7, the average surface thickness increased due to the immobilization of POEGMA-*b*-PNIPMAM (**4**) to the streptavidin layer. An increase of the thickness of 0.22 nm (± 0.05 nm) was calculated. In the following, the temperature dependent binding of the block polymer onto a streptavidin surface at 50°C was investigated. The previous measurements confirmed that at 50°C micelles are formed and thus the biotin end group should be hidden inside the micelle core preventing the selective immobilization of the block copolymer onto the streptavidin surface. For that, POEGMA-*b*-PNIPMAM (**4**) in PBS solution (1 g/L) was preheated to 50°C and at this temperature again three streptavidin immobilized surfaces were placed into the solution for 3 hours. After the reaction time, the glass slides were subsequently washed with 50°C warm PBS solution to remove any loosely bound polymer. Again, the increase of the surface thickness of each sample slides was analyzed by SPR. The surface thickness did not increase significantly with 0.01 nm (± 0.07 nm) as an average value of the three measurements (see figure 7). Obviously, while forming micellar structures, the block copolymer is not able to bind onto streptavidin. The slight increase of the film thickness can be explained by temperature fluctuations during the washing steps, as the polymer is able to bind to streptavidin at room temperature. This experiment demonstrated a

successful temperature controlled immobilization of a functional block copolymer onto a protein-coated surface. At room temperature, POEGMA-*b*-PNIPMAM (**4**) is molecularly dissolved and thus able to bind to streptavidin with its biotin end group. However, at 50°C the block copolymer forms micelles and the biotin end group is trapped inside the core of the micelles. As a consequence, the functional end group was not able to bind to streptavidin.

Conclusion

In summary, we have presented the synthesis of POEGMA-*b*-PNIPMAM (**4**) containing a bioactive biotin end group. The presence of the biotin end group could be proven by ^{1}H NMR and SPR measurements thus allowing the block copolymer to adsorb onto a streptavidin immobilized surface. As the block copolymer possessed two different thermo responsive blocks, a temperature dependent self-assembly was observed. The double temperature responsive behavior was successfully proven by temperature dependant NMR spectroscopy, DSC and cloud point measurements. Depending on temperature changes, the polymer was either soluble, formed micelles or even precipitated completely from aqueous solution. The formation of micelles was also demonstrated by DLS measurements, which measured a hydrodynamic diameter of 19 nm. Additionally, a temperature-controlled immobilization of POEGMA-*b*-PNIPMAM (**4**) with the biotin end group onto streptavidin surfaces was found. At 25°C the block copolymer was able to attach onto streptavidin while at 50°C, i.e. in the micellar form no adsorption could be observed. Concluding, we have demonstrated a suitable concept for a stimuli controlled binding of a smart block copolymer onto a protein immobilized surface. The synthetic approaches and the material properties presented here in this model system should be promising for further research and applications. For example, these materials might be a versatile system for a stimulus controlled binding of biopolymers onto a protein immobilized surface.

Acknowledgement

P. Glawion, R. Ragg, R. Forst and J. Podszuweit are acknowledged for support in the experimental work. This research was partly supported by WCU (World Class University) program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (R31-10013).

References

(1) Riess, G. *Prog. Polym. Sci.* **2003**, *28*, 1107-1170.

(2) Gohy, J. F.; Varshney, S. K.; Antoun, S.; Jerome, R. *Macromolecules* **2000**, 33, 9298-9305.

(3) Gohy, J. F.; Varshney, S. K.; Jerome, R. *Macromolecules* **2001**, *34*, 3361-3366.

(4) Topouza, D.; Orfanou, K.; Pispas, S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 6230-6237.

(5) Zhang, W. Q.; Shi, L. Q.; Miao, Z. J.; Wu, K.; An, Y. L. *Macromol. Chem. Phys.* **2005**, *206*, 2354-2361.

(6) Chen, D. Y.; Peng, H. S.; Jiang, M. *Macromolecules* **2003**, *36*, 2576-2578.

(7) Wu, C.; Niu, A. Z.; Leung, L. M.; Lam, T. S. *J. Am. Chem. Soc.* **1999**, *121*, 1954-1955.

(8) Antonietti, M.; Heinz, S.; Schmidt, M.; Rosenauer, C. *Macromolecules* **1994**, *27*, 3276-3281.

(9) Zhang, L. F.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777-1779.

(10) Zhang, L. F.; Eisenberg, A. *Science* **1995**, *268*, 1728-1731.

(11) Rao, J.; Xu, J.; Luo, S.; Liu, S. *Langmuir* **2007**, *23*, 11857-11865.

(12) Andre, X.; Zhang, M. F.; Müller, A. H. E. *Macromol. Rapid. Commun.* **2005**, *26*, 558-563.

(13) Arotcarena, M.; Heise, B.; Ishaya, S.; Laschewsky, A. *J. Am. Chem. Soc.* **2002**, 124, 3787-3793.

(14) Gohy, J. F.; Antoun, S.; Jerome, R. *Macromolecules* **2001**, *34*, 7435-7440.

(15) Rodriguez-Hernandez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026-2027.

(16) Butun, V.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1998**, *120*, 11818-11819.

(17) Liu, S. Y.; Armes, S. P. *Angew. Chem., Int. Ed.* **2002**, *41*, 1413-1416.

(18) Liu, S. Y.; Armes, S. P. *Langmuir* **2003**, *19*, 4432-4438.

(19) Liu, S. Y.; Weaver, J. V. M.; Tang, Y. Q.; Billingham, N. C.; Armes, S. P.; Tribe, K. *Macromolecules* **2002**, *35*, 6121-6131.

(20) Liu, S. Y.; Billingham, N. C.; Armes, S. P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2328-2331.

(21) McCormick, C. L.; Sumerlin, B. S.; Lokitz, B. S.; Stempka, J. E. *Soft Matter* **2008**, *4*, 1760-1773.

(22) Yan, Y.; Ji, W.; Chen, E.; Li, Z.; Liang, D. *Macromolecules* **2008**, *41*, 4908-4913.

(23) Hong, C.-Y.; You, Y.-Z.; Pan, C.-Y. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 4873-4881.

(24) Kim, K. H.; Kim, J.; Jo, W. H. *Polymer* **2005**, *46*, 2836-2840.

(25) Zhang, W.; Shi, L.; Wu, K.; An, Y. *Macromolecules* **2005**, *38*, 5743-5747.

(26) Zhang, W.; Jiang, X.; He, Z.; Xiong, D.; Zheng, P.; An, Y.; Shi, L. *Polymer* **2006**, *47*, 8203-8209.

(27) Gil, E. S.; Hudson, S. A. *Prog. Polym. Sci.* **2004**, *29*, 1173-1222.

(28) Plate, N. A.; Lebedeva, T. L.; Valuev, L. I. *Polym. J.* **1999**, *31*, 21-27.

(29) Maeda, Y.; Nakamura, T.; Ikeda, I. *Macromolecules* **2001**, *34*, 1391-1399.

(30) Idziak, I.; Avoce, D.; Lessard, D. *Macromolecules* **1999**, *32*, 1260-1263.

(31) Berndt, I.; Richtering, W. *Macromolecules* **2003**, *36*, 8780-8785.

(32) Lutz, J.-F. *J. Polym. Sci. Part A: Polym. Chem.* **2008**, *46*, 3459-3470.

(33) Lutz, J.-F.; Akdemir, Ö.; Hoth, A. *J. Am. Chem. Soc.* **2006**, *128*, 13046-13047.

(34) Lutz, J.-F.; Hoth, A. *Macromolecules* **2006**, *39*, 893-896.

(35) Lutz, J.-F.; Andrieu, J.; Üzgün, S.; Rudolph, C.; Agarwal, S. *Macromolecules* **2007**, *40*, 8540-8543.

(36) Lutz, J.-F.; Weichenhan, K.; Akdemir, Ö.; Hoth, A. *Macromolecules* **2007**, *40*, 2503-2508.

(37) Lutz, J.-F.; Börner, H. G.; Weichenhan, K. *Macromolecules* **2006**, *39*, 6376-6383.

(38) Skrabania, K.; Kristen, J.; Laschewsky, A.; Akdemir, Ö.; Hoth, A.; Lutz, J.-F. *Langmuir* **2007**, *23*, 84-93.

(39) Lutz, J.-F.; Stiller, S.; Hoth, A.; Kaufner, L.; Pison, U.; Cartier, R. *Biomacromolecules* **2006**, *7*, 3132-3138.

(40) Bontempo, D.; Maynard, H. D. *J. Am. Chem. Soc.* **2005**, *127*, 6508-6509.

(41) Lele, B. S.; Murata, H.; Matyjaszewski, K.; Russell, A. J. *Biomacromolecules* **2005**, *6*, 3380-3387.

(42) Mantovani, G.; Lecolley, F.; Tao, L.; Haddleton, D. M.; Clerx, J.; Cornelissen, J. J. L. M.; Velonia, K. *J. Am. Chem. Soc.* **2005**, *127*, 2966-2973.

(43) Oyane, A.; Ishizone, T.; Uchida, M.; Furukawa, K.; Ushida, T.; Yokoyama, H. *Adv. Mater.* **2005**, *17*, 2329-2332.

(44) Popescu, D. C.; Lems, R.; Rossi, N. A. A.; Yeoh, C.-T.; Loos, J.; Holder, S. J.; Bouten, C. V. C.; Sommerdijk, N. A. J. M. *Adv. Mater.* **2005**, *17*, 2324-2329.

(45) Tugulu, S.; Silacci, P.; Stergiopulos, N.; Klok, H.-A. *Biomaterials* **2007**, *28*, 2536-2546.

(46) Cai, T.; Marquez, M.; Hu, Z. *Langmuir* **2007**, *23*, 8663-8666.

(47) Wiss, K. T.; Krishna, O. D.; Roth, P. J.; Kiick, K. L.; Theato, P. *Macromolecules* **2009**, *42*, 3860-3863.

(48) Arotcarena, M.; Heise, B.; Ishaya, S.; Laschewsky, A. *J. Am. Chem. Soc.* **2002**, *124*, 3787-3793.

(49) Xu, J.; Luo, S.; Shi, W.; Liu, S. *Langmuir* **2006**, *22*, 989-997.

(50) Kotsuchibashi, Y.; Kuboshima, Y.; Yamamoto, K.; Aoyagi, T. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6142-6150.

(51) Kotsuchibashi, Y.; Yamamoto, K.; Aoyagi, T. *J. Coll. Interf. Sci.* **2009**, *336*, 67-72.

(52) Cao, Y.; Zhao, N.; Wu, K.; Zhu, X. X. *Langmuir* **2009**, *25*, 1699-1704.

(53) Sugihara, S.; Kanaoka, S.; Aoshima, S. *Macromolecules* **2005**, *38*, 1919-1927.

(54) Weaver, J. V. M.; Armes, S. P.; Butun, V. *Chem. Commun.* **2002**, *18*, 2122-2123.

(55) Berndt, I.; Pedersen, J. S.; Richtering, W. *J. Am. Chem. Soc.* **2005**, *127*, 9372-9373.

(56) Hua, F. J.; Jiang, X. G.; Zhao, B. *Macromolecules* **2006**, *39*, 3476-3479.

(57) Dimitrov, I.; Trzebicka, B.; Müller, A. H. E.; Dworak, A.; Tsvetanov, C. B. *Prog. Polym. Sci*. **2007**, *32*, 1275-1343.

(58) Heath, F.; Haria, P; Alexander, C. *AAPS Journal* **2007**, *9*, 235-240.

(59) Dai, S.; Ravi, P.; Tam, K. C. *Soft Matter* **2009**, *5*, 2513-2533.

(60) Cheng, C.; Wei, H.; Shi, B.-X.; Cheng, H.; Li, C.; Gu, Z.-W.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. *Biomaterials* **2008**, *29*, 497-505.

(61) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C *J. Am. Chem. Soc.* **2007**, *129*, 7145–7154.

(62) Heredia, K. L.; Bontempo, D.; Ly, T.; Byers, J. T.; Halstenberg, S.; Maynard, H. D. *J. Am. Chem. Soc.* **2005**, *127*, 16955–16960.

(63) Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *Macromol. Rapid Commun.* **2008**, *29*, 1172–1176.

(64) Chaiet, L.; Wolf, F. J. *Arch. Biochem. Biophys.* **1964**, *106*, 1–5.

(65) Weber, P. C.; Ohlendorf, D. H.; Wendoloski, J. J.; Salemme, F. R. *Science* **1989**, *243*, 85–88.

(66) Grunwald, C. *Zeitschr. Physik. Chem* **2008**, *222*, 789–821.

(67) Jonkheijm, P.; Weinrich, D.; Schröder, H.; Niemeyer, C. M.; Waldmann, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 9618–9647.

(68) Rusmini, F.; Zhong, Z.; Fejen, J. *Biomacromolecules* **2007**, *8*, 1775–1789.

(69) Göpel, W.; Heiduschka, P. *Biosens. Bioelectron.* **1995**, *10*, 853–883.

(70) Wang, X.; Liu, L.; Luo, Y.; Zhao, H. *Langmuir* **2009**, *25*, 744-750.

(71) Jochum, F. D.; zur Borg, L.; Roth, P. J.; Theato, P. *Macromolecules* **2009**, *42*, 7854-7862.

(72) Eberhardt, M.; Mruk, R.; Zentel, R.; Theato, P. *Eur. Polym. J.* **2005**, *41*, 1569-1575.

(73) Kessler, D.; Roth, P. J.; Theato, P. *Langmuir* **2009**, *25*, 10068-10076.

(74) Su, X.; Wu, Y.-J.; Robolek, R.; Knoll, W. *Langmuir* **2005**, *21*, 348-353.

(75) Roth, P. J.; Jochum, F. D.; Forst, F. R.; Zentel, R.; Theato, P. *Macromolecules* **2010**, *43*, 4638-4645.

(76) Theato, P. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6677-6687.

(77) Jochum, F. D.; Theato, P. *Macromolecules* **2009**, *42*, 5941-5945.

(78) Jochum, F. D.; Theato, P. *Polymer* **2009**, *50*, 3079-3085.

(79) Gauthier, M. A.; Gibson, M. I.; Klok, H.-A. *Angew. Chem., Int. Ed.* **2009**, *48*, 48-58.

(80) Roth, P. J.; Kessler, D.; Zentel, R.; Theato, P. *Macromolecules* **2008**, *41*, 8316–8319.

(81) Roth, P. J.; Kessler, D.; Zentel, R.; Theato, P. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 3118–3130.

(82) Roth, P. J.; Jochum, F. D.; Zentel, R.; Theato, P. *Biomacromolecules* **2010**, 11, 238-244.

(83) Boyer, C.; Bulmus, V.; Davis, T. P. *Macromol. Rapid Commun.* **2009**, *30*, 493–497.

(84) Boyer, C.; Liu, J.; Bulmus, V.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromolecules* **2008**, *41*, 5641–5650.

(85) Nicolas, J.; Mantovani, G.; Haddleton, D. M. *Macromol. Rapid Commun.* **2007**, *27*, 1083–1111.

(86) Heredia, K. L.; Maynard, H. D. *Org. Biomol. Chem.* **2007**, *5*, 45–53.

(87) Gonzalez, M.; Bagatolli, L. A.; Echabe, I.; Arrondo, J. L. R.; Argarana, C. E.; Cantor, C. R.; Fidelio, G. D. *J. Biol. Chem.* **1997**, *272*, 11288-11294.

Science cannot solve the ultimate mystery of nature.
And that is because, in the last analysis, we ourselves are part of nature
and therefore part of the mystery that we are trying to solve.

Max Planck

List of Publications

1. Jochum, F. D.; Theato, P. “Temperature and Light Sensitive Copolymers Containing Azobenzene Moieties Prepared via a Polymer Analogous Reaction”, *Polymer* **2009**, *50*, 3079-3085.

2. Jochum, F. D.; Theato, P. “Temperature- and Light-Responsive Polyacrylamides Prepared by a Double Polymer Analogous Reaction of Activated Ester Polymers”, *Macromolecules* **2009**, *42*, 5941-5945.

3. Jochum, F. D.; zur Borg, L.; Roth, P. J.; Theato, P. “Thermo- and Light-Responsive Polymers Containing Photoswitchable Azobenzene End Groups”, *Macromolecules* **2009**, *42*, 7854-7862.

4. Etika, K. C.; Jochum, F. D.; Theato, P.; Grunlan, J. C. “Temperature Controlled Dispersion of Carbon Nanotubes in Water with Pyrene-Functionalized Poly(*N*-cyclopropylacrylamide)”, *J. Am. Chem. Soc.* **2009**, *131*, 13598-13599.

5. Seo, J.; Schattling, P.; Lang, T.; Jochum, F. D.; Nilles, K.; Theato, P.; Char, K. “Covalently Bonded Layer-by-Layer Assembly of Multifunctional Thin Films Based on Activated Esters”, *Langmuir* **2010**, *26*, 1830-1836.

6. Roth, P. J.; Jochum, F. D.; Zentel, R.; Theato, P. “Synthesis of Hetero-Telechelic alpha,omega Bio-Functionalized Polymers”, *Biomacromolecules* **2010**, *11*, 238-244.

7. Schladt, T. D.; Shukoor, M. I.; Schneider, K.; Tahir, M. N.; Natalio, F.; Ament, I.; Becker, J.; Jochum, F. D.; Weber, S.; Kohler, O.; Theato, P.; Schreiber, L. M.; Sonnichsen, C.; Schröder, H. C.; Müller, Werner E. G.; Tremel, W. “Au@MnO Nanoflowers: Hybrid Nanocomposites for Selective Dual Functionalization and Imaging”, *Angew. Chem. Int. Ed.* **2010**, *49*, 3976-3980.

8. Roth, P. J.; Jochum, F. D.; Forst, F. R.; Zentel, R.; Theato, P. “Influence of End Groups on the Stimulus-Responsive Behavior of Poly[oligo(ethylene glycol) methacrylate] in Water”, *Macromolecules* **2010**, *43*, 4638-4645.

9. Jochum, F. D.; Roth, P. J.; Theato, P. “Thermoresponsive Polymers: From Different Functional End Groups to Block Copolymers”, *ACS Polymer Preprints* **2010**, *51*, 363-364.

10. Jochum, F. D.; Forst, F. R.; Theato, P. “PNIPAM Copolymers Containing Light-Responsive Chromophores: A Method Toward Molecular Logic Gates”, *Macromol. Rapid Commun.* **2010**, *31*, 1456-1461.

11. Etika, K. C.; Jochum, F. D.; Cox, M. A.; Schattling, P.; Theato, P.; Grunlan, J. C. “Nanotube Friendly Poly(*N*-isopropylacrylamide)”, *Macromol. Rapid Commun.* **2010**, *31*, 1368-1372.

12. Jochum, F. D.; Theato, P. “Thermo- and Light Responsive Micellation of Azobenzene Containing Block Copolymers”, *Chem. Commun.* **2010**, *46*, 6717-6719.

13. Jochum, F. D.; Roth, P. J.; Kessler, D.; Theato, P. “Double Thermo-Responsive Block Copolymers Featuring a Biotin End Group”, *Biomacromolecules* **2010**, *11*, 2432-2439.

Acknowledgement

The research presented in this thesis would not have been possible without the support and aid of different people and institutions.

First of all, I would like to thank my advisor Dr. XXX for the nice research topic, the valuable discussions, his suggestions and support during the last years. I gratefully thank Professor XXX for accepting me into his work group. I appreciate his own ideas towards my PhD work and the financial support.

I thank Professor XXX and his student XXX for the fruitful collaboration project.

I am very thankful for the support in experimental work done by XXX, XXX, XXX, XXX, XXX and XXX.

I like to thank all of my present and former colleagues of the AK Zentel / Theato for the great time and extraordinary atmosphere during my PhD work in Mainz. I am very proud to be a member of this nice research group. I especially thank XXX, XXX, XXX, XXX and XXX for scientific discussions in chemistry and beyond this field.

I would like to thank XXX for DSC measurements and for her caring commitment within the work group. I am thankful to XXX for giving me advices for the synthetic procedures. I highly appreciate the work of Mrs. XXX and Mrs. XXX in the secretariat. I am very thankful for their efforts in extending my contract.

I would like to thank the staff of the central analytic for several NMR spectroscopy and mass spectrometry measurements.

I acknowledge the Institute of Biophysics and the Institute of Pharmacy for allowing me access to instruments, which I needed for my PhD work.

I thank the German Academic Exchange Service (“DAAD”) for the financial support of my scientific conference trip to San Francisco to attend the ACS Spring Meeting 2010.

I am thankful to all people that contributed to this work, which I forgot to mention above.

Finally, I thank my parents, family and friends, and of course my girlfriend XXX for their support during my entire studies in Mainz.