Synthetic Routes toward Functional Block Copolymers and Bioconjugates via RAFT Polymerization

Dissertation

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Tag der mündlichen Prüfung:

„Wer glaubt, etwas zu sein,
hat aufgehört, etwas zu werden."

Philip Rosenthal

Contents

1 Abbreviations

AFM	atomic force microscopy/microscope
AIBN	2,2‘-azobisisobutyronitrile
Asp	aspartic acid
ATR	attenuated total reflection
ATRP	atom transfer radical polymerization
a.u.	arbitrary units
b	*block*
$BaSO_4$	barium sulfate
Boc	*tert*-butyloxycarbonyl
br	broad peak (for the description of bands/peaks in IR/NMR)
BSA	bovine serum albumin
t-Bu	*tert*-butyl
t-BuMA	*tert*-butyl methacrylate
c	concentration
C	cysteine
$CaCO_3$	calcium carbonate
calc	calculated
CD	circular dichroism
CHCA	α-cyano-4-hydroxycinnamic acid
$CHCl_3$	chloroform
CLTR	2-chlorotrityl chloride resin

CRP	controlled radical polymerization
CTA	chain transfer agent
CuBr	copper bromide
CuAAC	copper-catalyzed azide-alkyne cycloaddition
d	day(s) / doublet (for the description of peaks in NMR)
d_n	n-fold deuterated
DBU	1,8-diazabicyclo[5.4.0]undec-7-en
dd	doublet of doublets (for the description of peaks in NMR)
DEEA	2,2-diethoxyethyl acrylate
DEGMEMA	diethylene glycol methyl ether methacrylate
DLS	dynamic light scattering
DMA	1,2-dioxolan-2-ylmethyl acrylate
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
D_2O	deuterium oxide
DP	degree of polymerization
E	glutamic acid
e.g.	for example (lat. *exempli gratia*)
ESI-MS	electrospray ionization mass spectroscopy
Fmoc	N^{α}-9-fluorenylmethoxycarbonyl
FT	Fourier transformation
FWHM	full width at half maximum
G	glycine
Gly	glycine
GPC	gel permeation chromatography
HA1	*O*-heptylhydroxylamine
HA2	*O*-(3-hydroxypropyl)hydroxylamine
HEMA	hydroxyethyl methacrylate
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol

H_2O	water
Hz	Hertz
i.e.	that is (lat. *id est*)
IR	infrared
J	coupling constant
K	lysine
KTFA	potassium trifluoroacetate
l	path length
LCST	lower critical solution temperature
LiBr	lithium bromide
LiCl	lithium chloride
m	*meta*
m	mass / multiplet (for the description of peaks in NMR)
M	molecular weight (for small molecules) / mol/L (concentrations)
M_n	number average molecular weight
M_w	weight average molecular weight
MALDI	matrix assisted laser desorption/ionization
$MgSO_4$	magnesium sulfate
min	minute(s)
MMA	methyl methacrylate
MS	mass spectroscopy/spectrometer
MTMS	methyl trimethoxy silane
MW	molecular weight (for polymers)
n	*normal*
n_f	number of amino acid residues present in a peptide
NMP	nitroxide-mediated polymerization
NMR	nuclear magnetic resonance
o	*ortho*
OEGMEMA	oligoethylene glycol methyl ether methacrylate

p	*para*
P	proline
P_i*	propargating polymer chain
Pbf	2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PBS	phosphate buffered saline
PCP	PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer
PDI	polydispersity index = M_w / M_n
PEG	poly(ethylene glycol)
PFP-ACV	bis(pentafluorophenyl)azobis-(4-cyanovalerate)
PFP-CTA	pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate)
PMMA	poly(methyl methacrylate)
PMSSQ	poly(methyl silsesquioxane)
PNIPAAm	poly(*N*-isopropylacrylamide)
PS	poly(styrene)
PSS	"Polymer Standards Service" (Mainz)
q	quartet (for the description of peaks in NMR)
R	arginine
RAFT	reversible addition-fragmentation chain transfer
RAFT-Si	dithiobenzoic acid benzyl-(4-ethyltrimethoxysilyl)ester
RI	refractive index
RMS	root-mean-square deviation of the surface roughness
RP-HPLC	reverse-phase high performance liquid chromatography
rpm	rounds per minute
s	second(s) / singlet (for the description of peaks in NMR)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPPS	solid-phase peptide synthesis
t	tertiary (*tert*)
t	triplet (for the description of peaks in NMR)
t_R	retention time

T	temperature
T_m	melting temperature
TEA	triethylamine
TEM	transmission electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	trimethylsilane
TOF	time of flight
Trt	triphenylmethyl (trityl)
tt	triplet of triplets
UV(-vis)	ultraviolet (and visible light)
w	weak (for the description of bands/peaks in IR/NMR)
wt.-%	weight percent
z	charge

°	degree
δ	chemical shift
λ	wavelength
$[\theta]_{MRE}$	mean residue elipticity
Θ_a	advancing contact angle

2 Introduction

2.1 Motivation

In the last decades, one of the most rapidly emerging fields in materials science lies at the interface between polymer chemistry and biology. The combination of synthetic polymers with biological entities like peptides or proteins offers a multitude of possibilities to obtain fascinating new materials merging the properties of these two classes of materials and overcoming some of their limitations. Characteristics of natural peptides and proteins are, for example, the monodispersity in their amino acid sequence and size, hierarchical structure formation, biorecognition and binding, which can result in immunogenicity or even toxicity. In contrast, synthetic polymers are less uniform in structure and molecular weight, usually biologically inactive, and allow only for limited control over nanoscale structure. However, they allow for high flexibility in molecular architecture and mechanical properties, so that they can be designed to be biocompatible or resistant to enzymatic degradation, to name only a few of the different properties of these two classes of materials.

Moreover, this interdisciplinary topic is promoted by an increasing interest in materials suitable for pharmaceutical and diagnostic applications, tissue engineering, biomimetics, enzymatic catalysis and many other applications.[1-7] One of the first significant discoveries, which motivated further developments in the field of polymer-peptide-conjugates, was Davis' observation in the 1970s, that the

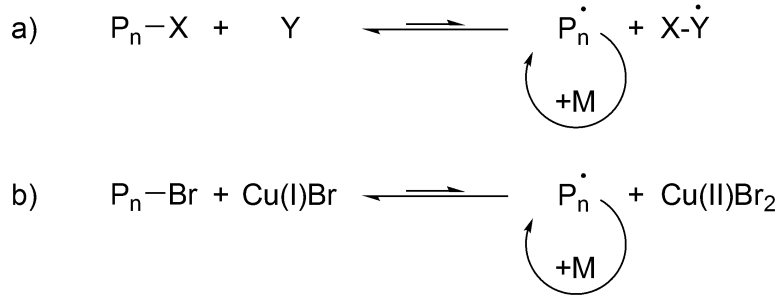
conjugation of poly(ethylenglycol) (PEG), nowadays referred to as PEGylation, to bovine serum albumin (BSA) and bovine liver catalase decreased the immunogenicity and increased the blood circulation time of these proteins ("stealth effect").[8-10] This work inspired the development of PEGylated proteins to the first class of polymer therapeutics in clinical use.[1,2] Besides bioconjugates, in which the biological component is responsible for pharmaceutical or other functions, many examples have been studied, in which peptide segments mediated the formation of superstructures on the nanometer scale.[4] Inspired by natural products such as spider silk, hybrid materials for example consisting of multiple poly(alanine) and PEG segments were designed.[11,12] This combination resulted in polymers with silk-like solid-state structures and very good, tunable mechanical properties, in which the tendency of the peptide segments to form antiparallel β-sheets led to discrete nanostructures. A third research area dealing with the development of binding strategies of peptides or proteins to synthetic polymers is the field of protein immobilization on surfaces for biochips, which play an important role as diagnostic tools in medicine and proteomics.[6] Therefor, lots of efforts were undertaken to improve polymeric coating materials, which allow for covalent protein binding, and deposition methods, which enable the simple preparation of stable coatings.

Despite a variety of synthetic approaches for PEGylation and non site-selective conjugation of polymers to peptides, it was the class of controlled radical polymerization (CRP) techniques, which paved the way for a broader applicability of the concept of bioconjugation on a larger variety of synthetic polymers and the synthesis of well-defined, highly sophisticated hybrid materials.[3-5,13-19] Motivated by promising advances on the field of functional polymers with reactive end-groups, polymer chemists are taking the challenge to improve these materials and to meet materials requirements in the future.

2.2 Controlled Radical Polymerization Techniques

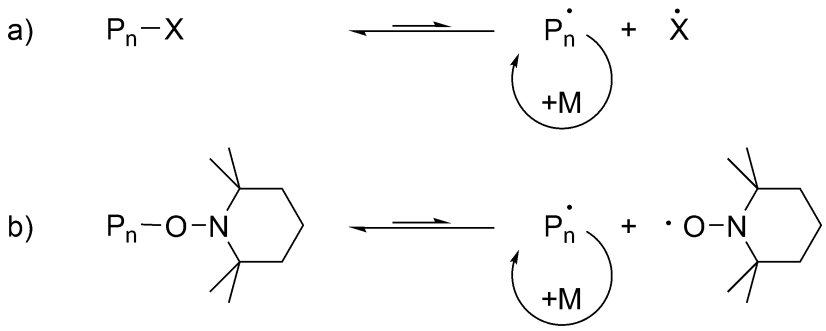
Traditionally, living polymerization techniques, and among these in particular anionic polymerization, have been the method of choice for the synthesis of polymers with controllable chain lengths, narrow molecular weight distributions and well-defined end-groups. Moreover, the "living" character of resulting polymers also allows for the synthesis of block copolymers via sequential addition of different monomers. However, due to the high reactivity of the anionic propagating chain end, the application of anionic polymerization is limited to aprotic solvents and only a small number of polymerizable monomers, especially if it comes to monomers with functional side groups. In addition, the necessary procedures are usually very elaborate. Free radical polymerization, in contrast, can be conducted under less demanding conditions and tolerates a huge variety of functional groups in the monomer structure, but results in polymers with broad molecular weight distributions and non-uniform end-groups due to the variety of possible termination and side reactions and thus provides no opportunity to prepare block copolymers. Controlled radical polymerization (CRP) techniques combine the advantages of the described methods under only a small increase in polydispersity of the obtained polymers compared to anionic polymerization and thus are a very useful compromise.[20-22]

The most popular CRP mechanisms are atom transfer radical polymerization (ATRP),[23-26] nitroxide-mediated polymerization (NMP),[27-31] and reversible addition-fragmentation chain transfer (RAFT) polymerization.[32-38] In ATRP and NMP, the most dominant termination reactions, namely disproportion and recombination, which both exhibit second order reaction rates with respect to the radical concentration, are suppressed by reduction of this concentration. This is realized by an equilibrium between the radical chain end and a dormant species, which itself is unable to propagate. In ATRP, this equilibrium is a reversible redox reaction catalyzed by a transition metal complex, usually based on a copper species (see **Scheme 1**).



SCHEME 1: a) General formulation of the equilibrium between dormant species and propagating polymer chain in ATRP, b) a classical example with a copper catalyst.

As its name already indicates, for NMP, a nitroxide is added to the polymerization, which undergoes a reversible addition to the radical chain end (see **Scheme 2**).



SCHEME 2: a) General formulation of the equilibrium between dormant species and propagating polymer chain in NMP, b) a classical example using TEMPO.

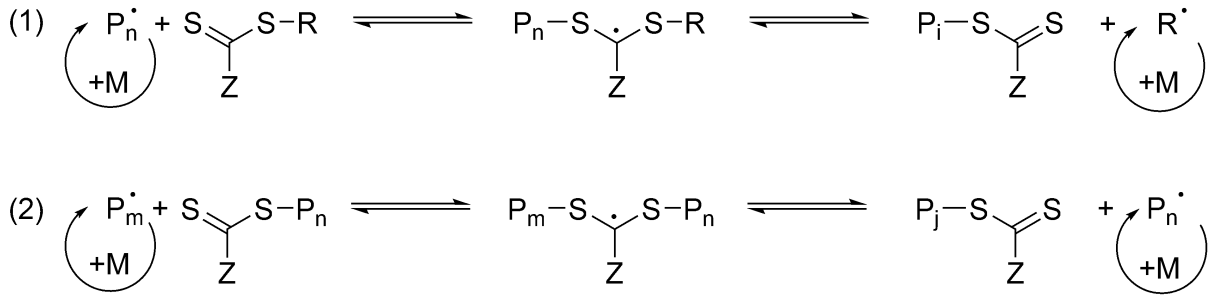
The RAFT process relies on a reversible chain transfer from one propagating chain end to another, which is mediated by a chain transfer agent (CTA).

All the three described methods enable the synthesis of a variety of homopolymers as well as block copolymers with controllable molecular weights and low polydispersities. However, the applicability of NMP suffers from the restricted number of polymerizable monomers, mostly styrenes and acrylates, and the disadvantage of ATRP lies in the use of the transition metal, which cannot be removed completely during the work-up of the product. It should be noted, that even traces of transition metals might have an influence on subsequent reactions or the structure formation and especially on the self-assembly behavior of peptides or other macromolecules, which can interact with the residual metal. Thus, in the

projects presented in the following, RAFT polymerization was chosen as the most suitable technique, which furthermore offers convenient ways to introduce functional groups at both the chain ends. A more detailed discussion of the RAFT mechanism is provided in the following.

2.2.1 Reversible Addition-Fragmentation Chain Transfer Polymerization

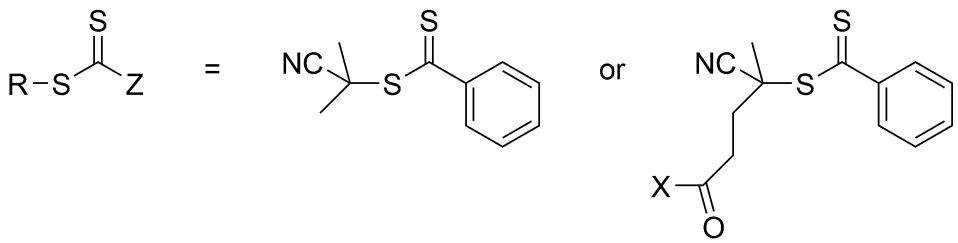
Since its invention in 1998,[32,33] the RAFT process rapidly gained popularity and found many applications not only because it enables the controlled synthesis of well-defined functional polymers, but also because it is a very convenient and easy method from the practical point of view. The only technical requirements are the use of pure solvents, the necessity to degas the reaction solution, a regular control over the reaction temperature, and the addition of an appropriate chain transfer agent to the solution of monomer and initiator. These three ingredients already allow for control over the resulting molecular weight and the polymer end-groups. The degree of polymerization is regulated by the monomer to CTA ratio (the initiator is only used in very small quantities as initial radical source), and the structure of the CTA determines the structure of the chain ends. The most frequently used CTAs are dithioesters, xanthates, dithiocarbamates, and trithiocarbonates.



SCHEME 3: Reversible Addition-Fragmentation Chain Transfer Polymerization.

Scheme 3 explains the RAFT mechanism using a dithioester, which was the CTA class of choice in the work presented in the following chapters. The initiator, usually

AIBN, is cleaved homolytically to produce the first radicals (isobutyronitrile radicals in the case of AIBN), which then start the first propagating polymer chains, P_n*, by reaction with a monomer. Besides the typical chain growth, these propagating chains can also react with the CTA by addition to the sulfur in the thiocarbonyl group of the dithioester (**Equation 1**, **Scheme 3**). The resulting intermediate carbon-centered radical can then undergo fragmentation, which either leads back to the propagating chain P_n* and the original CTA or liberates a macromolecular CTA and a radical leaving group R*. The new radical R* should be that stable, that the latter fragmentation is preferred. At the same time, it has to be reactive enough to reinitiate polymerization and produce another propagating chain, P_m*. That way, the original CTA is used up rapidly and an equilibrium between propagating chains and a symmetric intermediate carbon-centered radical is established (**Equation 2**, **Scheme 3**). The CTA should be chosen under consideration of the reactivity of the monomer, so that the described equilibrium favors the symmetric intermediate, but the chain transfer from one propagating species to the other as well as the propagation itself are fast, more precisely, orders of magnitude faster than termination. The choice of the R- and the Z-group is facilitated by the large number of existing reviews giving an overview of developed CTAs and recommended combinations of monomer and CTA.[35-40] Dithioesters with an isobutyronitrile group or a derivative of the same as R-group on the one hand, and a phenyl group as Z-group on the other hand, have proven to be suitable CTAs for the controlled polymerization of styrenes, (meth)acrylates, and most acrylamides (**Scheme 4**).



SCHEME 4: A versatile class of CTAs based on a dithioester.

Except for the small number of propagating chains started by the classical intitiator, the majority of the polymer chains obtained via the described mechanism exhibit the R-group as α-end-group and the dithioester including the Z-group as ω-end-group (**Figure 1**).

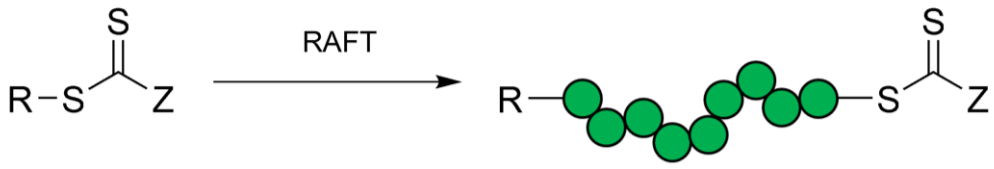


FIGURE 1: Polymers with functional end-groups via RAFT polymerization.

Therefore, it is possible to introduce functional end-groups at the α-chain end by using a CTA with a functionalized R-group. In this context, a functional group can either be a small molecule, for example a dye or an anchor group, or a reactive group, which allows for flexible conjugation before or after the polymerization. Using such a reactive handle, a CTA can also be linked to macromolecular entities, like inorganic polymers, peptides, or other classical polymers, resulting in a polymeric CTA, which can subsequently be used in a so-called "RAFTing-from" polymerization.

In general, the same considerations hold true for a functionalized Z-group with one significant difference: The Z-group is bound to the ω-chain end of the resulting polymer only via the labile dithioester linkage and thus, modification of the Z-group is not an appropriate mean to obtain a permanent functionalization. Nevertheless, the lability of the dithioester can be turned into an advantage. The dithioester itself offers diverse possibilities to convert the ω-end-group into a functional moiety after the polymerization (**Figure 2**). Very popular is the conversion of the dithioester into a terminal thiol group via aminolysis, hydrazinolysis or reduction.[41-46] However, these reactions often result in mixtures of thiols and disulfides, and in the case of poly(methacrylates), the formation of thiolactones via backbiting is observed predominantly.[44-46] In order to obtain better defined functional end-groups, the dithioester can for example be subjected to the following reactions: conversion into

an asymmetric disulfide via aminolysis in the presence of functional methane thiosulfonates,[47,48] hetero-Diels-Alder reactions with dienes (only for electron-deficient dithioesters as dienophiles),[49-51] and radical substitution with an excess of AIBN or one of its derivatives.[52-54]

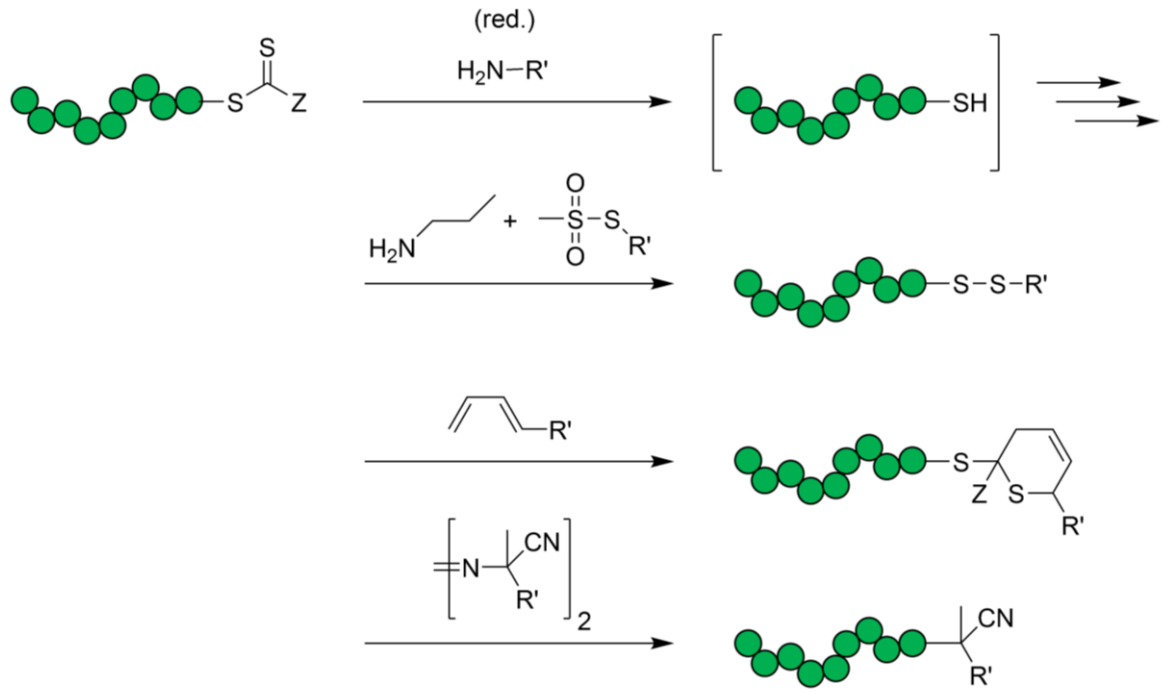


Figure 2: ω-End-group functionalization via conversion of the dithioester.

This variety of accessible functional end-groups turns the RAFT process into a versatile tool for the synthesis of block copolymers and further polymer architectures, like stars, macrocycles, polymer brushes, inorganic-organic hybrids, and bioconjugates.[36,39,55,56]

2.3 Block Copolymer Synthesis Using the RAFT Process

Due to their interesting self-organization behavior,[57] block copolymers gain a lot of attention in materials science and are applied in diverse research areas such as optoelectronics[58-61] and biomedicine.[1-5] In bulk, the different polymer blocks undergo phase-separation resulting in geometric morphologies depending on the volume

fraction of the two (or more) components of the block copolymer which turns them into useful precursors for the preparation of nanostructured functional materials. Amphiphilic block copolymers can self-assemble in solution into supramolecular structures like micelles or vesicles, structures which are of special interest because of their capability to encapsulate small molecules like dyes or drugs. Moreover, if one or even two blocks consist of a stimuli-responsive polymer, which changes its polarity and thus its solubility upon application of an external stimulus like irradiation, external fields, a change in temperature, salt concentration or pH, such supramolecular compartments can be triggered by that external stimulus to release their content.[62-65]

The development of controlled radical polymerization (CRP) techniques like the RAFT process facilitated the synthesis of well-defined, sophisticated block copolymers by two alternative pathways. On the one hand, the incorporation of functional end-groups allows for conjugation of independently polymerized building blocks (“grafting-to”). On the other hand, it is possible to use one polymer block as macromolecular chain transfer agent in the polymerization of the second block (“grafting-from” / ”RAFTing-from”).

Both approaches have their advantages and disadvantages. When using a macromolecular CTA in the polymerization of a second polymer block, the first challenge is the quantitative reinitiation. If not all of the macromolecular CTA initiates a polymerization of the second monomer, the resulting product will contain a mixture of block copolymer and homopolymer. Also, the polymerization of the second monomer can not always be controlled as accurately as the one of the first polymer, so that a narrow molecular weight distribution is not necessarily obtained. However, the isolation of the final block copolymer is usually achieved straightforward by precipitation, because the raw product consists only of the desired product and small molecular impurities such as unconverted monomer, if the reinitiation was successful. Depending on the nature of the two building blocks,

the characterization of the block copolymer, more precisely of the second polymer block, can be another challenge. For example, the determination of the degree of polymerization and the polydispersity index (PDI) of the second block via gel permeation chromatography (GPC) can be complicated due to the fact that the hydrodynamic volume does usually not increase linearly with the molecular weight of the second block. In extreme cases, the obtained block copolymer might even exhibit a smaller hydrodynamic volume than the first polymer block. For many applications however, it is desirable to know the exact composition of the employed materials. Especially, if polymers shall be approved for pharmaceutic use, they need to be characterized very well, but also if they are used for the preparation of structured materials, for example, the block length ratio plays a major role. This is an advantage of the "grafting-to" approach, in which all the building blocks are polymerized individually, and thus usually in an easily controllable reaction, and can be characterized independently prior to conjugation. It also allows for uncomplicated combination of building blocks of diverse nature, which cannot be synthesized via the same polymerization mechanism. A drawback of this method is that the conjugation efficiency suffers very often from steric hindrance, which increases with the molecular weight of the building blocks. Besides leading to a lower conversion, it also complicates the purification of the product, which contains macromolecular impurities, namely the unreacted homopolymers, in the case of non-quantitative conversion. In summary, both described approaches have their place and can be used as powerful tools for the synthesis of block copolymers, if chosen under consideration of the nature of the building blocks as well as the designated application of the particular product.

In the following, preparative examples for the application of the RAFT process in block copolymer synthesis will be given after some general remarks.

As mentioned before, to prepare block copolymers via the "RAFTing-from" approach means to employ one of the building blocks as macromolecular CTA in the

polymerization of another block. Such a macromolecular CTA is either simply obtained as the regular product of the first polymerization, if this was already performed as "living" polymerization using a RAFT agent, or it can be synthesized via conjugation of a small molecular CTA with a reactive group to any kind of macromolecule independent of the mechanism employed for the synthesis of this first block. Alternatively, a dithioester moiety could also be built up step by step via organic synthesis at one end-group of a macromolecule, which itself was not synthesized via RAFT polymerization. However, the diverse synthetic approaches developed for this purpose strongly depend on the nature of the first block and therefore are not depicted here.

The use of a functional CTA also represents the most convenient way toward the application of a polymer, which was produced via the RAFT process, in polymer conjugation ("grafting-to"). As already described, any functionality contained in the R-group of a CTA is later on incorporated into the α-end-group of the generated polymer, given that it does not interfere with the polymerization itself. That way, reactive handles for the conjugation to other polymer blocks can be installed at the α-end-group of a polymer. Additionally, the ω-end-group resulting from the RAFT process can be employed as or be converted into a reactive handle for polymer conjugation.

One of the most popular reactions in the field of polymer modification nowadays is the copper-catalyzed 1,3-dipolar cycloaddition of azides to alkynes,[66-72] which also allows for the conjugation of polymeric building blocks to each other. Therefor, several CTAs with either azide or terminal alkyne groups have been developed.[73-76] The efficiency of polymer conjugation via this copper-catalyzed azide-alkyne cycloaddition (CuAAC) was demonstrated in the conversion of poly(vinylacetate), which was synthesized with an azide-functionalized CTA, with poly(styrene) resulting from a RAFT polymerization using a CTA with a trimethylsilyl-protected alkyne group,[73] for example.

In order to synthesize block copolymers with a reversible linkage, two CTAs, one with an aldehyde functionality and one with a hydroxylamine group were employed in RAFT polymerizations. That way, for instance, poly(styrene), poly(methyl methacrylate), and poly(isoprene) with an aldehyde or a hydroxylamine moiety at the α-end-group were obtained and successfully conjugated to each other.[77] Micelles formed from this kind of block copolymers could be triggered to decompose by the addition of a small molecular hydroxylamine, confirming the dynamic character of the oxime linkage between the two polymeric building blocks.

As described in these examples, one can theoretically introduce a variety of reactive handles into the α-end-groups of polymers using functional CTAs, as long as they are stable under the conditions used for the RAFT polymerization, but not too many successful conjugation reactions between two macromolecular entities have been described yet via this particular approach. However, some more functional CTAs can be considered as promising candidates for polymer conjugation,[35-40,53,78-83] some of them even enable the synthesis of heterotelechelic polymers with two different reactive chain ends. For example, a variety of heterotelechelic polymers were synthesized using a trithiocarbonate-based CTA with an azide in the R-group and a Z-group containing a pyridyl disulfide.[82] With these polymers with orthogonally reactive end-groups in hand, the preparation of ABC triblock copolymers is imaginable via combination of CuAAC chemistry with thiol-disulfide exchange. Moreover, more complex architectures such as an AB_2 triblock copolymer could possibly also be designed by using a CTA with a branched R-group, which exhibits two thiol-reactive pyridyl disulfide groups.[83]

The variety of functional CTAs is enormous and could probably be expanded without limits, but usually the synthesis of a new CTA is rather time-consuming, and the stability and applicability in a radical polymerization also has to be tested for each and every new CTA. Therefore, a universal CTA would be highly desirable, which could, depending on the particular application, be converted easily into other

CTAs prior to polymerization or enable simple and efficient end-group modification after polymerization.

As discussed in chapter 2.2.1, the dithioester ω-end-group can also be utilized for the synthesis of block copolymers. For its application in chain extension ("RAFTing-from"), the ω-end-group does not need to be modified at all and the first polymer block is simply used as macromolecular CTA in another RAFT polymerization. Since basically the same theoretical and practical details as already described for RAFT homopolymerizations in chapter 2.2.1 hold true for such a sequential RAFT polymerization and as many textbooks[38,57] address this topic extensively, it is not discussed here in detail.

Most examples utilizing the ω-end-group for polymer conjugation require an end-group modification after polymerization to turn the dithioester into a reactive handle. For instance, the reductive aminolysis of a trithiocarbonate ω-end-group using 2-ethanolamine in combination with tributylphosphine was demonstrated to yield thiol-terminated poly(*N*-isopropylacrylamide) and poly(styrene), briefly PNIPAAm-SH and PS-SH,[84] which generally allow for polymer conjugation via diverse reaction types. Sumerlin and coworkers presented the conversion of such a PNIPAAm-SH into its maleimide-terminated analog via base-catalyzed Michael addition to bis(maleimido)diethyleneglycol and its subsequent conjugation to another PS-SH.[84]

Recently, a new class of CTAs with electron-withdrawing Z-groups was shown to result in polymers with ω-end-groups, which could directly be used as dienophile in hetero-Diels-Alder reactions with diene-functionalized polymers. The fastest reaction was observed with polymers with a cyclopentadiene end-group.[50] However, the number of monomers, which can be polymerized using these novel CTAs, is still limited, and the synthesis of cyclopentadiene-terminated polymers usually involves a nucleophilic substitution with sodium cyclopentadienide, which again excludes several monomers with reactive side groups.

A more universal approach toward functional ω-end-groups is the radical substitution[52] of the dithioester or trithiocarbonate via treatment with an excess of functional derivatives of AIBN.[53,54] That way, a protected maleimide was introduced to the ω-end-group of PNIPAAm, which allowed for reactions with thiol-terminated macromolecules after thermal deprotection (120°C, vacuum) of the maleimide.[54]
With the described methods for α- and ω-end-group modification on the basis of the RAFT process in hand, also heterotelechelic polymers can be obtained,[82,85,86] which are promising candidates for the preparation of ABC triblock copolymers. However, only very few examples have been realized.

2.4 Application of the RAFT Process in Bioconjugation

Different methods for the synthesis of polymer-peptide- and polymer-protein-conjugates have been developed in the recent years due to an increasing interest in applications like drug delivery, tissue engineering, diagnostics, biomimetics, or enzymatic catalysis. Mainly the advances in the field of controlled radical polymerization techniques combined with easy, powerful reaction types summarized under the concept of "click chemistry"[67] facilitated the improvement of the synthetic strategies in terms of efficiency and site-selectivity.[5,13,15,18,19] Among the different CRP techniques, the RAFT process is the most versatile and suitable for bioconjugation, because it does not require the use of a transition metal catalyst, but is applicable for the polymerization of a large variety of functional monomers, in contrast to ATRP or NMP.[17] Thus, this chapter is focused on the synthesis of bioconjugates via RAFT polymerization.
In general, the same synthetic strategies as described for the synthesis of block copolymers can be employed in order to obtain linear bioconjugates. The two main approaches are the so-called *in situ* polymerization ("grafting-from") from the biological entity, which herein acts as a macromolecular CTA, and the coupling

("grafting-to") of polymers with functional end-groups to peptides or proteins. However, the preparation of hybrid copolymers entails additional challenges. One of them arises from the different properties of these two classes of materials, which are different solubilities and thermal stability, for example. Hence, it can be tedious to find reaction conditions appropriate for both building blocks. Another challenge is the need for bioorthogonal conjugation reactions allowing for efficient, but also site-selective coupling of either the CTA or the polymer to the biomolecule, which should not be disturbed by this reaction in terms of its structure and bioactivity, in the ideal case. In the following, some selected examples of successful reaction conditions will be discussed, also demonstrating the advantages and disadvantages of the two possible approaches.

First examples of the "grafting-from" approach involved peptide-based CTAs, which were synthesized via solid-phase peptide synthesis (SPPS) followed by a coupling reaction, in which a CTA was attached to the N-terminus of the peptide prior to cleavage from the resin.[87] In general, the CTA could be conjugated to the peptide via a functional group, which is a carboxyl group in most examples, contained either in the R- or the Z-group of the CTA. However, the R-group was used mostly for linkage to the peptide. In both cases, at least one equivalent of the CTA should be used in order to avoid aminolysis of the thiocarbonyl moiety. An alternative method to circumvent the possibility of thioamide formation was a 2-step procedure for the build-up of a CTA moiety at the N-terminus of the peptide: First, 2-bromopropionic acid was conjugated to the amino end-group, and after cleavage of the peptide from the resin, dithiobenzoic acid was used to substitute the bromide resulting in a dithioester.[87-89] These peptide-based CTAs were successfully applied in the RAFT polymerization of diverse monomers such as *n*-butyl acrylate, NIPAAm, and oligo(ethylene glycol) acrylate, after which the side groups of the peptide segments could be deprotected, yielding polymer-peptide diblock copolymers.[87,88,90] This approach could be expanded onto the preparation of triblock copolymers via sequential polymerization of two different monomers using the same CTA or by

using a PEG-functionalized resin for SPPS.[89] Börner and coworkers used the "grafting-from" approach for the preparation of polymer-peptide-conjugates with varying degree of polymerization in the poly(*n*-butyl acrylate) block and found that a percentage of only 3.5wt.-% peptide in the overall structure were sufficient for peptide-driven self-assembly of these hybrids into fibrillar microstructures.[88]

While RAFT polymerizations from small protected peptides can be performed under standard polymerization conditions (for example at temperatures above 60°C using thermal initiators) and in organic solvents, *in situ* polymerizations from native proteins require more peptide-friendly conditions such as lower temperatures and aqueous solvent mixtures in order to conserve structural integrity and thus biological activity of the protein.

After the first controlled radical polymerization from a defined site in a protein had already been performed via ATRP using biotin-functionalized initiators,[91] which were bound to streptavidin, a thiol-reactive CTA could be conjugated to a specific cysteine residue (Cys-34) on the surface of the protein BSA and subsequently be used for the RAFT polymerization of oligo(ethylene glycol) acrylate.[81] Therefor, a trithiocarbonate-based CTA with a pyridyl disulfide in the Z-group was used, which was reacted site-specifically with the free thiol under disulfide exchange. Due to insufficient solubility of this CTA in pure water, the polymerization had to be conducted in a mixture of water and DMF, and it was initiated via γ-radiation in order to avoid high temperatures. That way, polymer blocks of molecular weights up to roughly 30 000 g/mol (MW determined after reductive cleavage of the linkage between polymer and protein) with PDIs between 1.2 and 1.8 were obtained, and it was hypothesized that the higher PDIs were caused by steric hindrance of the RAFT end-group by the growing polymer chain. In addition to the desired polymer-protein-conjugates, homopolymer was found in the product mixture. In order to overcome several of the described drawbacks of this first approach, the same authors developed a water-soluble CTA bearing a short PEG segment in the R-group and allowing for polymerization under more protein-friendly conditions (i.e. initiation

using a room-temperature initiator in a purely aqueous system (phosphate buffer)).[80] This CTA was bound to BSA via the same method and successfully used for RAFT polymerization of NIPAAm, for example. Testing of the esterase activity of BSA confirmed that neither the polymerization conditions nor the presence of a protein-bound polymer block affected the structural integrity of the protein. Sumerlin and coworkers presented the functionalization of the same cysteine residue of BSA with a CTA containing a maleimide moiety in the R-group.[92] The esterase activity of the PNIPAAm-BSA-conjugates, which were prepared via RAFT polymerization from this functionalized protein at 26°C in phosphate buffer, was found to be controllable by changes in temperature. More precisely, the conjugates exhibited full biological activity at room temperature, i.e. below the LCST of PNIPAAm, and reduced activity at 40°C, i.e. above the LCST of PNIPAAm, and this transition was reversible over more than 5 temperature cycles. Moreover, the LCST behavior of the polymer block could be utilized for separation of unfunctionalized protein from the raw product via precipitation of the desired product, which is especially valuable considering the fact, that native BSA exhibits only 0.45 free thiol groups per molecule at its surface, which can be modified chemically.

As discussed before, steric hindrance of the CTA by the growing polymer chain can occur during polymerization, especially if the CTA is coupled to the peptide or protein via the Z-group, while conjugation of the CTA via the R-group results in a terminal thiocarbonyl group during polymerization and thus should allow for better chain transfer. However, the labile thioester linkage cannot be utilized to cleave the final polymer block easily from the protein, in this case, due to its position at the chain end. Hence, if characterization of the obtained polymer block is desired, harsh conditions (reductive treatment over 5 days) allowing for protein degradation need to be applied. It is a general disadvantage of the "grafting-from" approach, that information about the degree of polymerization and PDI of the polymer block are not accessible straightforward and it often requires cleavage of the product only for analysis. Further, not in all examples, full control over the radical polymerization

was achieved, resulting in broader molecular weight distributions, possibly undefined end-groups, and the presence of homopolymer in the raw product. Despite these limitations of the *in situ* polymerization, it should be mentioned, that an exactly defined molecular weight and low PDI are not necessarily needed for every imaginable application of this type of bioconjugates. For instance, these characteristics do not play a major role, when an enzyme is functionalized with a stimuli-responsive polymer for enzyme recovery after application in a catalytic process, while a bioconjugate designed for pharmaceutical application might need to be characterized in more detail in order to be approved for clinical use.

In contrast, the "grafting-to" approach offers the possibility to combine building blocks with well-defined and more exactly accessible properties. Sumerlin and coworkers also developed a synthetic route for the preparation of a PNIPAAm-BSA-conjugate via this alternative approach.[93] PNIPAAm with an azide at the α-end-group, a molecular weight of about 16 300 g/mol, and a PDI of 1.06 was produced via RAFT polymerization mediated by a CTA with an azide-bearing R-group. This reactive polymer was converted in a copper-catalyzed azide-alkyne cycloaddition (CuAAC) using copper sulfate and ascorbic acid in an aqueous solution with an alkyne-functionalized BSA, which was obtained from the reaction of propargyl maleimide with the free thiol group at the surface of the protein. The product showed similar thermo-responsive behavior as the PNIPAAm-BSA-conjugate prepared via RAFTing-from confirming the covalent linkage between the two building blocks and also allowing for separation of the desired product from unreacted protein. However, non-converted homopolymer cannot easily be separated from this hybrid material because of the similar thermo-responsive behavior, and in contrast to the conjugation of a small molecule to the protein, the reaction between two macromolecular entities cannot necessarily be assumed to reach full conversion, even when working with efficient types of reaction. This can be explained by steric hindrance, which usually limits the "grafting-to" approach to

polymers with lower molecular weights in comparison to the grafting-from approach.

A variety of other polymers with one reactive end-group have been synthesized (compare chapters 2.2 and 2.3), which could in general also enable conjugation to peptides or proteins but will not be discussed here in detail. Instead, first examples of telechelic polymers, which allow for conjugation of two peptide or protein units to one polymer block with relatively simple means, will be given to point out a strong advantage of the „grafting-to“ approach over „grafting-from“.

Maynard and coworkers polymerized NIPAAm using a bis(trithiocarbonate)-CTA for mediation and subsequently converted the two resulting trithiocarbonate end-groups with an excess of a functional AIBN derivative.[54] With the help of this diazo-compound, protected maleimide end-groups were introduced at both chain ends, which were subsequently reacted with a thiol group of a lysozyme after thermal deprotection via retro-Diels-Alder reaction. SDS-PAGE and mass spectroscopy confirmed the formation of protein-PNIPAAm-protein-conjugates (21%) besides the main product consisting of PNIPAAm and one protein unit (79%) and thus demonstrated the success of the general concept, while gradient cation exchange allowed for the isolation of the pure triblock copolymer. The same group also prepared PNIPAAm with a biotin-functionalized α-end-group derived from the R-group of the employed CTA and a maleimide ω-end-group obtained via the same radical substitution method as described in the previous example.[85] The application of this heterotelechelic polymer as linker between two different proteins, namely streptavidin and BSA, was demonstrated starting with the thioether formation between the free cystein residue accessible at the surface of BSA with the maleimide, which was followed by ligation of the biotin end-group by streptavidin. SDS-PAGE verified the presence of conjugates containing both proteins, but due to multiple biotin binding sites available on one streptavidin, it has to be assumed that more than one biotin was bound to most of the streptavidin units.

Taking together these two examples and the advances described in the previous chapters, the combination of functional CTAs with radical substitution of the thiocarbonyl end-group(s) by treatment with functional derivatives of AIBN compiles a promising toolbox for bioconjugation. Nevertheless, it should be mentioned that the presented studies do not yet represent universally applicable synthetic routes, but rather specific binding strategies restricted to model proteins, namely biotin-binding proteins, like avidin and streptavidin, and BSA, very often only chosen because of the convenience of a single free thiol on its surface. Hence, the exploration of further, ideally more universal types of conjugation reactions is highly desirable.

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3 Aims and Objectives

The scope of this dissertation is to develop synthetic strategies suitable for the preparation of bioconjugates consisting of peptides or proteins and synthetic polymers. In this context, the method of choice to obtain well-defined and precisely functionalizable polymer building blocks is controlled radical polymerization, and among the available methods, reversible addition-fragmentation chain transfer (RAFT) polymerization should be employed. The RAFT process qualifies as a versatile tool for the preparation of block copolymers and polymer-peptide-conjugates, because it is compatible with a large variety of functional groups and thus allows for introduction of peptide-reactive groups to side- and end-groups of the resulting polymers.

Considering the existing literature on available polymer and bioconjugation strategies, more universal approaches for polymer-peptide-coupling would be desirable. Therefore, synthetic routes should be explored herein, which address amine groups commonly present at distinct and sometimes even multiple sites in most peptide sequences (at least one precisely positioned amine group in each peptide is available at the N-terminus). In the context of this dissertation, two amine-reactive functionalities, namely activated esters and aldehydes, should be investigated as candidates for the linkage to biological entities. Both allow for efficient conversion with amine groups without the need for a catalyst. However, since aldehydes can interfere with the polymerization mechanism and tend to crosslink, their protected analogs, the class of acetals, should be used during the

polymerization step. The reactive aldehyde can then be obtained afterwards via acidic deprotection. Activated esters, in contrast, are the activated analogs of carboxylic acids and as such can easily react with different kinds of nucleophiles, but preferentially with amines. They are stable at neutral and acidic pH and under conditions of radical polymerizations, and thus can be employed in the RAFT process as they are, possibly even in combination with acetal moieties. In this thesis, the pentafluorophenyl ester should be used, because it offers a very attractive feature in addition to high reactivity. This is the possibility to monitor its conversion via ^{19}F NMR spectroscopy, a very sensitive method even at low concentrations, which turns it into an ideal technique to potentially survey the reaction of a single end-group in a macromolecule. Via the controlled radical polymerization using functional chain transfer agents, these moieties can be precisely incorporated at the end-groups of, for example, reactive or stimuli-responsive polymers.

With the described tools selected, the goal of this dissertation should be to develop and test methods for efficient functionalization of polymer chain ends with reactive handles, which should subsequently be used for coupling reactions with other polymers and peptides. Depending on the nature of the respective building blocks, appropriate reaction conditions and procedures need to be found, which also allow for exclusive isolation of the desired product.

Given that a universal and thus modular coupling chemistry can be developed, smart bioconjugates, for example including stimuli-responsive polymers, should be designed. Identifying interesting hybrid structures as well as exploring their special properties or potential applications is of further interest.

A next step will be the preparation of reactive surface coatings. Coating materials with functional groups recently attract increasing attention because of their applicability in the production of diagnostic devices like biochips for protein immobilization. In this context, polymeric materials would be desirable, which enable a robust attachment of the coating to the substrate and easy modification of the surface properties as well as protein binding via reactive groups on the surface.

4 Results

4.1 Synopsis

As outlined under „Aims and Objectives“ (chapter 3), the goal of this dissertation was to find synthetic routes for the preparation of well-defined polymer-peptide-conjugates and materials for peptide- or protein-reactive surface coatings utilizing the RAFT process.

First of all, this requires efficient strategies for the incorporation of reactive end-groups into a polymer structure enabling polymer and bioconjugation. The use of a functional chain transfer agent (CTA) for RAFT polymerization represents a nearly quantitative method for the functionalization of the chain ends and diverse CTAs with functional R-groups can be synthesized. However, a universal CTA would be highly desirable, which allowed for conversion into other CTAs via a simple reaction prior to polymerization or provided an efficient possibility for end-group modification after polymerization. Hence, on the one hand, the synthesis of many functional CTAs could be facilitated and, on the other hand, a single polymer precursor could be employed in the exploration of diverse coupling reactions suitable for polymer or bioconjugation only via simple variation of its end-group.

A chain transfer agent with an activated ester moiety in the R-group, namely pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA), which

was developed in a cooperative project,[1] is an ideal candidate for this purpose. The activated ester in the PFP-CTA or in the α-end-group of the final polymer can be reacted easily with amines yielding stable amide bonds and thus allows for the introduction of a variety of reactive handles as well as for direct conversion with amine groups of proteins or other biomolecules. Moreover, it offers an additional advantageous feature. Its conversion can be monitored easily via ^{19}F NMR spectroscopy, which is more sensitive than most of the other classical characterization methods. This is especially valuable for polymer analogous reactions at a single end-group of a macromolecule, where other methods sometimes reach their detection limit due to the low concentration of the group of interest.

The use of this PFP-CTA for the implementation of the described concept of flexible CTA design and end-group modification as tools for polymer conjugation will be presented in chapter 4.2. As a proof of principle, a CTA bearing an alkyne in the R-group is synthesized and applied in RAFT polymerizations of different methacrylates. The alkyne group can be used as a reactive handle for polymer conjugation via 1,3-dipolar cycloaddition between alkynes and azides. For example, the copper-catalyzed cycloaddition of an alkyne-functionalized poly(diethylene glycol methyl ether methacrylate) to an azide-functionalized poly(*tert*-butyl acrylate) is demonstrated. In addition, the introduction of an azide as well as an alkyne functionality to the α-end-group of poly(methacrylates) after their polymerization is shown.

In chapter 4.3, the direct conversion of pentafluorophenyl ester α-end-groups with amine groups of peptides is applied for the synthesis of well-defined bioconjugates. More precisely, stimuli-responsive polymers, namely poly(diethylene glycol methyl ether methacrylate) and poly(oligoethylene glycol methyl ether methacrylate), are conjugated successfully to one or two end-groups of a collagen-like peptide sequence yielding di- or triblock copolymers, respectively. The structural integrity of the peptide segments in the polymer-peptide-conjugates, the self-assembly behavior of

[1] Roth, P. J.; Wiss, K. T.; Zentel, R.; Theato, P. *Macromolecules* **2008**, *41*, 8513-8519.

these hybrids in solution, and the stimuli-responsive character of the observed superstructures are investigated.

As a tool for the functionalization of the ω-end-group, an AIBN derivative with two activated ester groups can be used for the radical substitution of the dithioester end-group of polymers obtained via RAFT polymerization. Such a functional diazo compound with two pentafluorophenyl ester moieties was also presented by Roth et al.,[1] and herein its application for bioconjugation is demonstrated in chapter 4.4. In combination with the described method for introduction of the same activated ester group to the α-end-group homotelechelic polymers can be prepared. On the basis of such a homotelechelic poly(diethylene glycol methyl ether methacrylate), a triblock copolymer with two peptide blocks is synthesized.

A final study explores materials for functional surface coatings suitable for the immobilization of peptides or proteins, as well as polymer-peptide-conjugates. Using a macromolecular CTA based on poly(methyl silsesquioxane)[2] for the RAFT polymerization of different acrylate monomers with acetal side groups, inorganic-organic hybrid copolymers can be obtained, which enable the preparation of stable coatings via spin-coating followed by thermally induced crosslinking. As the preparation and functionalization of comparable surfaces with activated ester groups was already demonstrated previously,[3] it was a logical consequence to investigate acetal groups, which exhibit orthogonal reactivity to activated esters. Further, via the variation of the protecting group, acetals with different stabilities toward acidic deprotection can be realized. With these materials and the diversity of reactive handles in hand, stable coatings with independently addressable reactive moities can be prepared. Their applicability for surface functionalization and modification of the surface properties is demonstrated in chapter 4.5 via conversion with a variety of primary amines after acidic deprotection.

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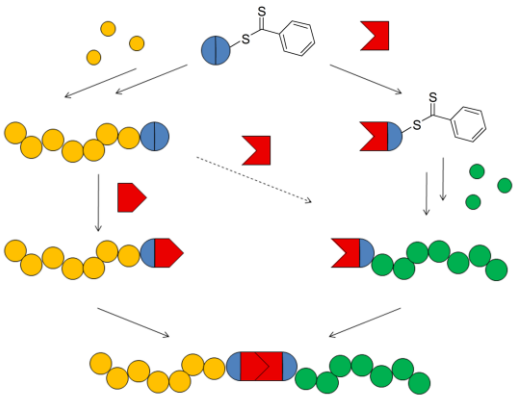
[2] Kessler, D.; Theato, P. *Macromolecules* **2008**, *41*, 5237-5244.

[3] Kessler, D.; Metz, N.; Theato, P. *Macromol. Symp.* **2007**, *254*, 34-41.

4.2 Facilitating Polymer Conjugation via Combination of RAFT Polymerization and Activated Ester Chemistry

Wiss, K. T.; Theato, P. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *submitted.*



Abstract

The synthesis of block copolymers via polymer conjugation of well-defined building blocks offers excellent control over the structures obtained, but often several coupling strategies need to be explored in order to find an efficient one depending on the building blocks. To facilitate the synthesis of polymers with adjustable functional end-groups for polymer conjugation, we report on the combination of activated ester chemistry with RAFT polymerization using a chain transfer agent (CTA) with a pentafluorophenyl ester (PFP-CTA), which allows for flexible functionalization of either the CTA prior to polymerization or the obtained polymer after polymerization. Different polymethacrylates, namely PMMA, P(*t*-BuMA) and PDEGMEMA, were synthesized with an alkyne-CTA obtained from the aminolysis of the PFP-CTA with propargylamine, and the successful incorporation of the alkyne moiety could be shown via ^{1}H and ^{13}C NMR spectroscopy and MALDI TOF MS. Further, the reactive α-end-groups of polymers synthesized using the unmodified PFP-CTA could be converted into azide and alkyne end-groups after polymerization, and the high functionalization efficiencies could be demonstrated via successful coupling of the

resulting polymers via CuAAC. Thus, the PFP-CTA allows for high combinatory flexibility in polymer synthesis facilitating polymer conjugation as useful method for the synthesis of block copolymers.

Introduction

A variety of strategies for the precise synthesis of block copolymers and more complex polymer architectures have been developed in the last years due to the increasing attention these materials attracted. Block copolymers, for example, exhibit interesting self-assembly properties in bulk and solution which allow for versatile applications in a broad range of research areas from optoelectronics[1-4] to biomedicine.[5-9] Controlled radical polymerization (CRP) techniques facilitated the synthesis of well-defined, sophisticated polymer architectures by either the consecutive polymerization of different monomers because of the living character of CRP techniques ("grafting-from") or the incorporation of functional end-groups for polymer conjugation ("grafting-to").

An important advantage of the convergent „grafting-to" approach is not only the exact control over chain length and polydispersity of the building blocks utilizing different polymerization techniques but rather their independent synthesis and characterization prior to conjugation. This is for example highly valuable in the case of materials for pharmaceutical use, which require thorough characterization prior to application. Furthermore, this strategy allows for high combinatory flexibility when it comes to variation of the building blocks, and it represents an easy way to combine polymer blocks of monomers, which cannot be polymerized via the same mechanism and are thus of different nature, i.e. this strategy enables easily the formation of polymer-peptide conjugates or conjugation of synthetic polymers with other biological entities. Several synthetic approaches have been developed, for example the use of functional initiators for atom transfer radical polymerization (ATRP)[10-13] or functional chain transfer agents (CTA) for reversible addition-fragmentation chain transfer (RAFT)[14-20] polymerization, which both result in polymers with functional

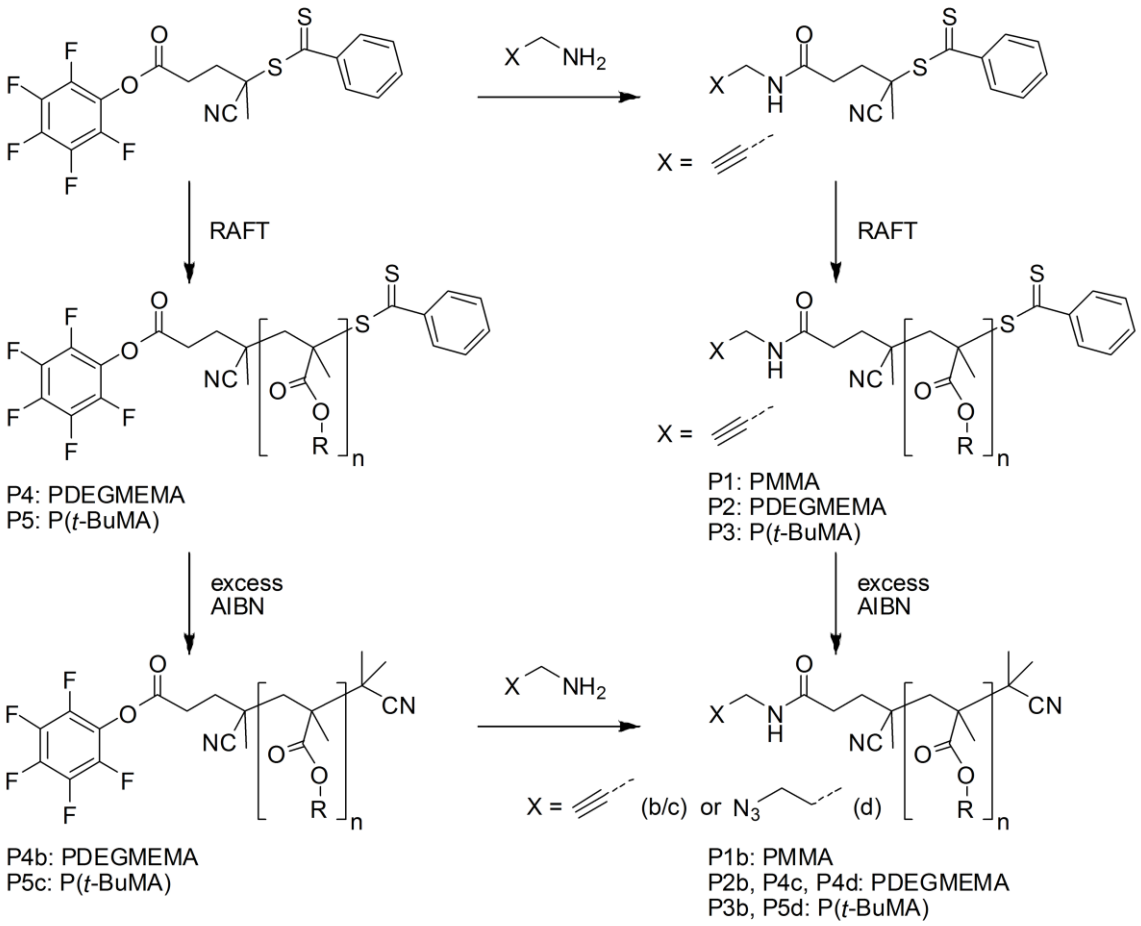
α-end-groups.[21-23] Unfortunately, polymer conjugation reactions tend to suffer from low efficiencies, which complicate the isolation of the product from the macromolecular building blocks. Finding highly efficient coupling reactions would facilitate the purification, especially when using an excess of one building block so that the other component should be completely consumed during the reaction and only one macromolecular reactant would be left with the product. So far, trying out different coupling reactions usually meant to synthesize the same polymer with different functional end-groups which requires time-consuming syntheses of diverse functional initiators or chain transfer agents for the controlled polymerization.

In this paper, we report on the combination of activated ester chemistry with RAFT polymerization that provides a versatile tool for the synthesis of well-defined polymers with adjustable functional end-groups. Utilizing a CTA with a pentafluorophenyl ester enables very efficient polymer conjugation or bioconjugation to amine-terminated polymeric building blocks, but also allows for flexible functionalization of either the chain transfer agent prior to polymerization or the obtained polymer after polymerization.

Results and Discussion

A crucial step on the way toward more efficient and versatile polymer conjugation strategies is a flexible and reliable end-group functionalization that enables the incorporation of diverse reactive groups and thus allows for high combinatory flexibility. Using functional CTAs for RAFT polymerization represents an easy way to introduce a functional group to the α-end-group of a well-defined polymer, but synthesis of such functional CTAs can be time-consuming and not all reactive moieties will withstand the polymerization.[26] The CTA pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA) features an activated ester group that allows selective reaction with amines and thus permits to overcome both of these limitations.[24,27-31] The PFP-CTA can either be converted into other functional CTAs by simple aminolysis of the activated ester prior to polymerization or can yield

polymers with a reactive α-end-group that can be further functionalized easily and efficiently after RAFT polymerization of desired monomers (**Scheme 1**). In the following, both synthetic pathways will be demonstrated and an example for successful polymer conjugation of the obtained polymers will be shown.



SCHEME 1: Overview of the different synthetic pathways toward functional α-end-groups for polymer conjugation.

Synthesis of an alkyne-CTA 2-cyano-5-oxo-5-(prop-2-ynylamino)pentan-2-yl benzodithioate (alkyne-CTA) was obtained from the reaction of propargylamine with the PFP-CTA, which was synthesized according to a procedure published previously.[24] Briefly, azobis(4-cyanovaleric acid) was reacted in dry dichloromethane overnight with pentafluorophenol in the presence of trifluoroacetic anhydride and 2,6-lutidine, yielding bis(pentafluorophenyl) azobis(4-cyanovalerate) after precipitation in cold hexane. Under stirring in degassed ethyl acetate at 80°C for 16 h,

the latter was reacted with dithiobenzoic acid disulfide (obtained from the reaction of phenylmagnesium chloride with carbon disulfide followed by conversion of the dithiobenzoic acid into the disulfide with a mixture of iodine and potassium iodide). The dark red CTA was isolated via column chromatography using dichloromethane for the first run and a mixture of dichloromethane and petrolether (6:4) for the second run.

The aminolysis of the PFP-CTA with propargylamine was conducted in dry THF, under addition of N^1,N^1,N^8,N^8-tetramethylnaphthalene-1,8-diamine as a non-nucleophilic auxiliary base at room temperature. To avoid aminolysis of the dithioester moiety, a slight excess of the PFP-CTA was used (propargylamine : PFP-CTA = 1 : 1.15). After neutralization with 1M hydrochloric acid and extraction with chloroform, the unconverted reactant could easily be separated from the product via quick column chromatography, which was facilitated by the color change observed during conversion. The product could be obtained in quantitative yield with respect to the quantity of propargylamine used.

TABLE 1: Polymers obtained from RAFT polymerization.

P#	Polymer	CTA	m(M[a])[b]	m(CTA)[b]	M_n[c]	M_w[c]	PDI	Yield
1	PMMA	C≡C	556	50	2.3k	2.7k	1.19	71%
2	PDEGMEMA	C≡C	2578	216	3.9k	4.6k	1.17	99%
3	P(*t*-BuMA)	C≡C	676	50	3.7k	4.2k	1.13	60%
4	PDEGMEMA	PFP	2000	236	2.8k	3.1k	1.09	97%
5	P(*t*-BuMA)	PFP	2500	224	2.3k	2.5k	1.10	34%

[a] M = Monomer; [b] Masses given in mg; [c] Molecular weights given in g/mol, obtained from GPC in THF

Synthesis of polymers with an alkyne α-end-group The alkyne-functionalized CTA could be employed in a standard RAFT polymerization (in dry dioxane, 20 h at 70°C under argon) to produce well-defined polymethacrylates, namely PMMA, P(*t*-

BuMA) and PDEGMEMA, in good yields exhibiting the alkyne-group at their α-chain end (see **Table 1**).

The incorporation of the alkyne end-group was verified via ^{1}H and ^{13}C NMR spectroscopy. As an example, the ^{13}C NMR of alkyne-functionalized PMMA (P1) is shown in **Figure 1** and the ^{1}H NMR of the same polymer will be discussed later (**Figure 2**). Besides the characteristic peaks of the MMA repeat units, the ^{13}C NMR showed signals representing both desired end-groups resulting from the alkyne-CTA. The signals corresponding to the carbon atoms in the triple bond could be found at 79.26 ppm and 71.74 ppm. Another interesting observation was the fact that the last repeat unit at the ω-end-group of the polymer caused weak peaks (here labeled with 2, 8, 10, and 16) with slightly different chemical shifts in comparison to the other repeat units, which is due to the proximity to the dithioester end-group.

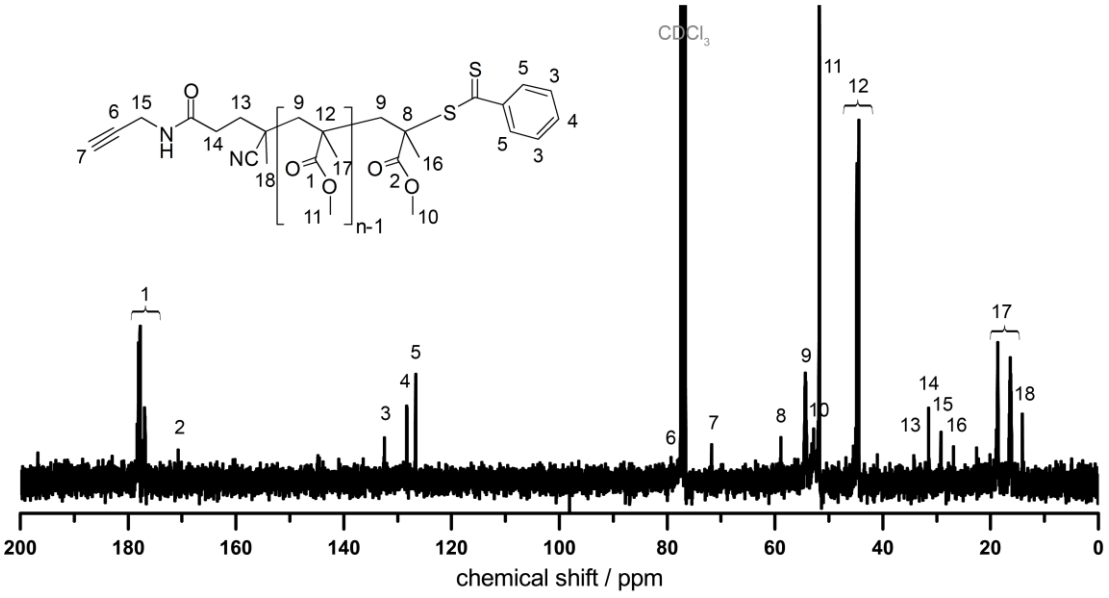


FIGURE 1: ^{13}C NMR of PMMA with alkyne α-end-group (P1).

These polymers P1-3 would already be appropriate candidates for a polymer conjugation via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) without need for post-polymerization functionalization, however, to make sure that no side reactions or undesired interactions of the ω-end-group with the catalyst occur, the

dithioester was first converted into an inert isobutyronitrile group by excessive treatment with AIBN following the procedure described by Perrier et al..[32] Briefly, polymers with a dithioester end-group resulting from the RAFT process were reacted with a 20-fold excess of AIBN in dry dioxane at 80°C for 2.5 h. After evaporation of most of the solvent, the products were isolated and purified via precipitation from a cool mixture of diethyl ether and hexane (1:1). The stability of the alkyne end-group toward the described radical substitution was shown on the example of alkyne-functionalized PMMA (P1b).

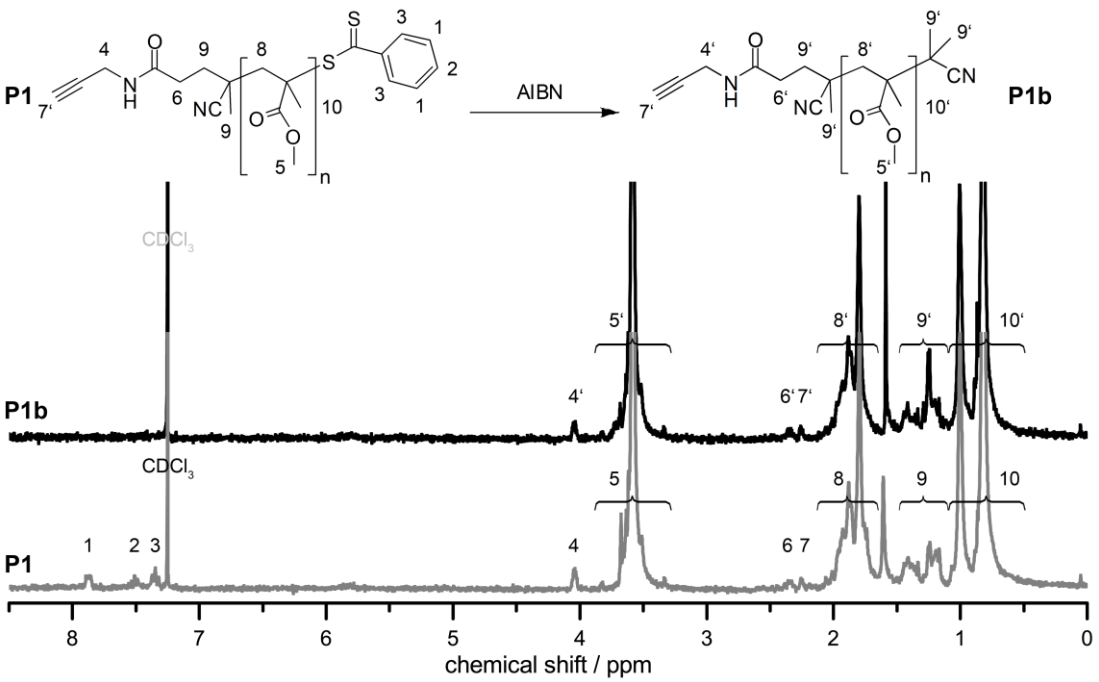


FIGURE 2: ^{1}H NMR of PMMA with alkyne α-end-group before (P1) and after (P1b) substitution of the dithioester ω-end-group.

Figure 2 shows the ^{1}H NMR spectrum of P1b in comparison to P1. Besides the expected signals of the polymer backbone and side-groups, the ^{1}H NMR showed a broadened peak at 4.05 ppm, which could be assigned to the methylene group between the amide bond and the alkyne group in the α-end-group. The broadened peak at 2.26 ppm corresponded to the terminal proton at the triple bond. And the disappearance of the aromatic signals of the phenyl group in the dithioester affirmed the substitution of the ω-end-group. ^{13}C NMR spectroscopy also confirmed the

structure of PMMA and the desired end-groups. Both the signals representing the carbon atoms of the alkyne group could still be found at 79.29 ppm and 71.77 ppm after the end-group conversion, while the aromatic signals of the phenyl end-group as well as the slightly shifted signals of the last repeat unit at the ω-end disappeared. As expected, the signal at 14.06 ppm increased in intensity, now representing three instead of only one methyl group next to a nitrile group. Further, the correct molecular weights corresponding to the desired end-group functionalities could be found in the MALDI TOF mass spectrum (see **Figure 3**), and the distance between the individual peaks (∅=101 g/mol) matched the mass of a methyl methacrylate repeat unit. Thus, there is sufficient evidence for the successful incorporation of the alkyne group using the functionalized CTA and its stability toward the radical substitution of the dithioester end-group.

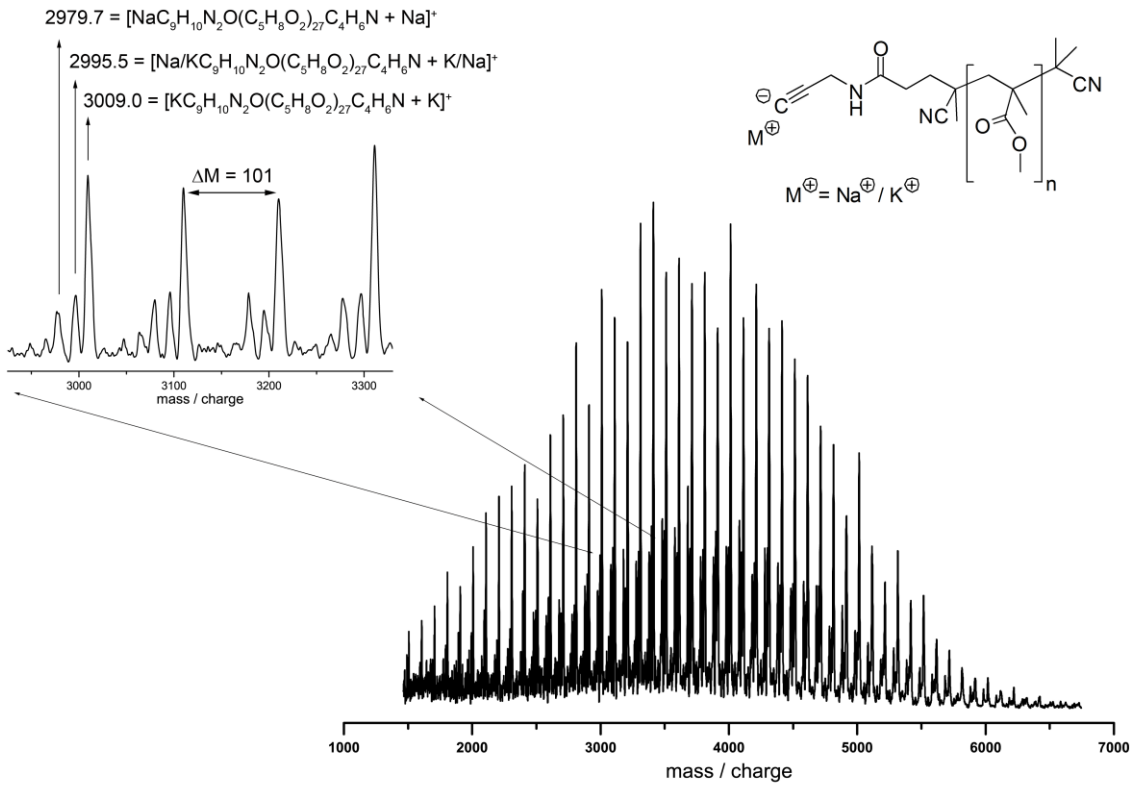


FIGURE 3: MALDI TOF mass spectrum of PMMA with alkyne α-end-group and isobutyronitrile ω-end-group (P1b).

Synthesis of polymers with a pentafluorophenyl ester α-end-group An alternative way toward functional α-end-groups, is the direct use of the PFP-CTA for the RAFT

polymerization of methacrylates, which was conducted under the same conditions as the polymerization with the alkyne-CTA described above. The resulting polymers exhibited the activated ester in their α-end-group, which could be detected in ^{19}F NMR spectroscopy at -152.59, -157.49 and -161.97 ppm and could be converted into a variety of functional end-groups via reaction with primary amines after the polymerization. Due to the high sensitivity of ^{19}F NMR, the conversion of this reactive pentafluorophenyl ester end-group can be monitored unusually well even in the case of relatively long polymers with a molecular weight of 20 000 g/mol and higher. Since several possible side reactions of the dithioester as end-group of polymethacrylates under the conditions of such an aminolysis are known,[33-36] this ω-end-group was substituted by an isobutyronitrile group in a reaction with 20-fold excess of AIBN analog to the one demonstrated above for the alkyne-terminated polymers and already described elsewhere.[31]

Conversion of the activated ester end-group of PDEGMEMA into an alkyne In order to convert the activated ester end-group of PDEGMEMA (P4b) into an alkyne group after polymerization, the polymer was reacted with propargylamine in dry THF at 35°C in the presence of triethylamine as auxiliary base. After 2 hours and isolation of the product by precipitation, the alkyne-functionalized PDEGMEMA (P4c) was obtained with a yield of 92%. In ^{1}H NMR spectroscopy, the shift of the signals representing the methylene group next to the converted carbonyl group from 2.84 ppm (characteristic of the methylene group next to the activated ester) to 2.32 ppm (characteristic of the methylene group next to an amide) and the signal of the terminal proton at the triple bond at 2.24 ppm clearly indicated the successful synthesis of the alkyne end-group. The complete disappearance of the signals corresponding to the pentafluorophenyl ester in ^{19}F NMR confirmed full conversion of the activated ester.

In the case of the alkyne end-group, the use of the prefunctionalized CTA and the conversion of the activated ester end-group after polymerization are exchangeable and yield the same polymer. For less stable functional groups like an azide, for

example, it might be more suitable not to introduce this group until the last step to avoid side reactions during the polymerization.[26] Therefore, the synthesis of azide-functionalized PDEGMEMA and P(*t*-BuMA) from polymers with activated ester end-groups will be demonstrated in the following.

Conversion of the activated ester α-end-group of P(*t*-BuMA) and PDEGMEMA into an azide For the transformation of the pentafluorophenyl ester into an azide end-group, 1-azido-3-aminopropane was synthesized from 3-chloropropylamine as previously published elsewhere.[25] The end-group conversion itself followed the same procedure as described above for the reaction with propargylamine, here using 1-azido-3-aminopropane. P(*t*-BuMA) (P5d) was isolated via precipitation from a mixture of methanol and deionized water (1:1) and obtained with a yield of 59%, while PDEGMEMA (P4d) was precipitated from a mixture of diethyl ether and hexane (1:5) and obtained with a yield of 70%. In both cases, the signals corresponding to the activated ester disappeared completely in ^{19}F NMR and the characteristic shift of the signals representing the methylene group next to the converted carbonyl group also indicated the success of these reactions in ^{1}H NMR. Signals caused by both the methylene group next to the azide and the one next to the nitrogen of the amide could be observed in the spectrum of P(*t*-BuMA) between 3.43 ppm and 3.25 ppm, while they are overlapped by signals of the polymer side chains in the case of PDEGMEMA. However, both converted polymers exhibited the characteristic band of an azide group at 2098 cm^{-1} in the IR spectra. These measurements together are a considerable indication for the successful conversion of the activated ester group into an azide end-group, however, both cannot proof a quantitative incorporation of 1-azido-3-aminopropane. An elegant way to demonstrate the ultimate efficiency of the end-group functionalization is a successful polymer conjugation reaction. In order to guarantee high conjugation efficiency, one building block was used in excess. As a consequence full consumption of the building block used in lower quantity can be achieved, if this component is fully functionalized.

Polymer conjugation via copper(I)-catalyzed azide-alkyne-cycloaddition As an example and proof of concept, the conjugation of alkyne-terminated PDEGMEMA (P2b) which resulted from a RAFT polymerization utilizing the alkyne-CTA, to azide-terminated P(*t*-BuMA) (P5d), which was synthesized via the PFP-CTA and the subsequent conversion of the α-end-group with 1-azido-3-aminopropane, was performed. A 1.5-fold excess of PDEGMEMA was used over P(*t*-BuMA), and both polymers were mixed with the solid catalyst copper bromide with a little amount of dry THF. An excess of PDEGMEMA was used because it can easily be separated from the raw product due to its solubility in cold water, as will be shown below. The polymer mixture was degassed separately from a solution of PMDETA in dry THF, which was also degassed and then transferred to the first Schlenk flask, so that the complete reaction mixture could then be stirred under inert gas for 24 h at 40°C. After filtration of the green suspension over silica gel and evaporation of the THF, the raw product was obtained. For further purification, it was redissolved in THF and precipitated from cold Milli-Q water, which was supposed to dissolve unreacted PDEGMEMA homopolymer. **Figure 4** shows the GPC elugrams of the building blocks, the raw product and the purified product without residual PDEGMEMA, indicating a successful polymer conjugation reaction as well as an efficient separation of the resulting diblock copolymer from the unconverted PDEGMEMA, which was used in excess. The little shoulder to lower molecular weights in the elugram of the purified product represents a small quantity of unconverted P(*t*-BuMA), which was calculated by peak deconvolution to be only 2 wt.-% of the total product. Such a small percentage of non-functional homopolymer is to be expected when working with the RAFT technique due to the small percentage of AIBN, which is added as radical source.[37-38] The complete consumption of azide-functionalized P(*t*-BuMA) is further supported by the complete disappearance of the azide band in IR spectroscopy. Overall, this conjugation example demonstrates the high functionalization degree of the P(*t*-BuMA), which could be obtained via aminolysis of the activated ester end-group.

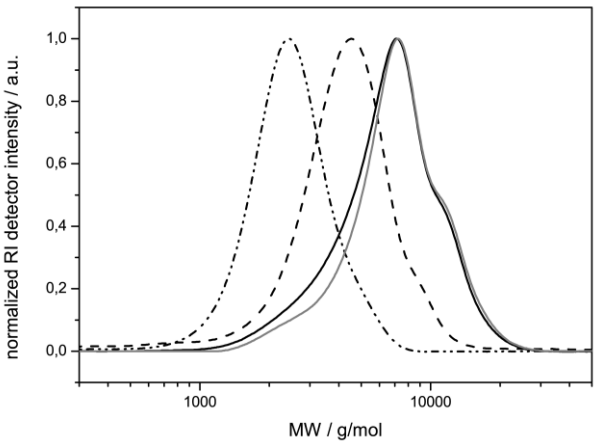


FIGURE 4: GPC traces of reactants, namely P(t-BuMA) (· ·) and PDEGMEMA (- - -), raw product (black) and product (grey) from polymer conjugation via CuAAC.

Experimental

Materials All chemicals and solvents were commercially available and used as received unless described otherwise in the following. Dioxane and tetrahydrofurane (THF) were distilled from sodium / potassium, and dichloromethane was dried via distillation with calcium hydride. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized from diethyl ether. Diethylene glycol methyl ether methacrylate (DEGMEMA), methyl methacrylate (MMA) and *tert*-butyl methacrlyate (*t*-BuMA) were purified by distillation in vacuum. Prior to its use as catalyst, copper(I) bromide (CuBr) was heated up in concentrated acetic acid and then isolated by filtration from the hot solvent and washed with methanol.

Instrumentation ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 300 MHz FT-NMR spectrometer, and ^{19}F NMR spectra on a Bruker AC 376 MHz FT-NMR spectrometer. Gel permeation chromatography (GPC) was used to determine molecular weights and polydispersity indices (PDI), M_w/M_n, of polymeric samples with respect to polystyrene standards. Therefore, a GPC set-up was used consisting of the following compounds: a Jasco PU-1580 pump, a Jasco AS-1555 autosampler, MZ-Gel-SDplus columns (10^2, 10^4 and 10^6 Å^2), a Jasco RI-1530 refractive index detector, and a Jasco UV-1575 UV/vis detector. Infrared (IR) spectra were measured on a Bruker Vector 22 FT-IR spectrometer with ATR unit. Electrospray ionization

mass spectroscopy (ESI-MS) was conducted on a QTof Ultima 3 Micromass Waters mass spectrometer, and elementary analysis on a Vario EL Cube by Elementar. Molecular weight distributions were measured on a Kratos Analytical Shimadzu AXIM-CFR MALDI TOF (matrix assisted laser desorption/ionization time of flight) mass spectrometer. The sample and the matrix (dithranole) were independently dissolved in chloroform (10 mg / mL) and the two solutions mixed to equal parts. This mixture (2 μL) was placed onto a multistage target plate, allowed to dry, and then the same volume of a cationization agent solution (1 mg / mL KTFA in methanol) was added.

Synthesis of 2-cyano-5-oxo-5-(prop-2-ynylamino)pentan-2-yl benzodithioate (alkyne-CTA) Pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate), briefly PFP-CTA, (50 mg, 0.11 mmol), which was synthesized according to a previously published procedure,[24] was dissolved in dry THF (0.8 mL) in a reaction tube equipped with a stir bar and a septum. Propargylamine (5.3 mg, 0.096 mmol) and N^1,N^1,N^8,N^8-tetramethylnaphthalene-1,8-diamine (24 mg, 0.11 mmol) dissolved in THF (0.2 mL) were added and the reaction mixture was stirred for 90 minutes at room temperature. Afterwards, 1M hydrochloric acid (4 mL) was added and the product was extracted with chloroform (4 mL). Subsequently, the aqueous phase was extracted twice with chloroform and the combined organic phases were dried over magnesium sulfate. The solvents were evaporated under reduced pressure and the raw product was purified by column chromatography with petrolether and ethyl acetate (1:1) as eluent. After evaporation of the solvents, the red product (30 mg, 0.095 mmol, 99%) was obtained. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.89 (d, 2H, J = 7.6 Hz, *o*-Ar), 7.56 (t, 1H, J = 7.6 Hz, *p*-Ar), 7.39 (t, 2H, J = 7.6 Hz, *m*-Ar), 5.83 (s, br, 1H, N*H*), 4.06 (dd, 2H, J^3 = 5.2 Hz, J^4 = 2.6 Hz, CH_2), 2.69 - 2.37 (m, 4H, CH_2CH_2), 2.23 (t, 1H, J = 2.6 Hz, C*H*), 1.93 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 219.09 (*C*(=S)S); 170.15 (*C*(=O)N); 144.47 (*ipso*-*C*H); 133.07 (*m*-*C*H); 128.59 (*p*-*C*H); 126.67 (*o*-*C*H); 118.69 (*C*≡N); 79.22 (*C*≡CH); 71.79 (C≡*C*H); 46.00 (*C*(CH_3)CN); 33.89 (C(=O)$CH_2$$CH_2$); 31.55 (C(=O)$CH_2$); 29.35 ($CH_2$-C≡CH); 24.18 ($CH_3$). ^{19}F NMR (376 MHz, $CDCl_3$): no residual

^{19}F signal. Elem. anal. calcd. for $C_{16}H_{16}N_2OS_2$: C, 60.72; H, 5.11; N, 8.85; S, 20.27; found: C, 60.15; H, 5.14; N, 8.47; S, 19.93. ESI-MS (*m*/*z*): calcd. for $C_{16}H_{16}N_2OS_2$: 316.07; found: 317.10 $[M + H]^+$, 339.07 $[M + Na]^+$, 355.05 $[M + K]^+$.

General procedure for RAFT polymerizations In a dry Schlenk tube equipped with a stir bar, monomer, CTA (1 equivalent) and AIBN (0.1 equivalent) were dissolved in freshly distilled dioxane (2 mL per 1 g monomer). The reaction mixture was degassed by three freeze-pump-thaw cycles and the flask refilled with argon. It was then stirred in a preheated oil bath at 70°C for 20 h. For isolation of the polymer, the product was precipitated three times in cool hexane in the case of poly(methyl methacrylate), PMMA, and poly(diethylene glycol methyl ether methacrylate), PDEGMEMA, or a mixture of methanol and deionized water (1:1) in the case of poly(*tert*-butyl methacrylate), P(*t*-BuMA). Explicit quantities used, obtained molecular weights, polydispersities and yields are given in **Table 1**.

P1: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.87 (m, 2H, *o*-C*H* (phenyl end-group)); 7.51 (m, 1H, *p*-C*H* (phenyl end-group)); 7.35 (m, 2H, *m*-C*H* (phenyl end-group)); 4.04 (w, 2H, CH_2-C≡CH (end-group)); 3.58 (br, 3nH, OCH_3); 2.34 (w, 2H, C(=O)CH_2 (end-group)); 2.26 (w, 1H, C≡C*H* (end-group)); 2.10 – 1.65 (br, 2nH, CH_2 (backbone)); 1.50 – 1.10 (w, 5H, C(=O)CH$_2$CH_2(CH_3) (end-group)); 1.08 – 0.50 (br, 3nH, CCH_3 (backbone)); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 178.32 (*C*(=O)O); 178.04 (*C*(=O)O); 177.74 (*C*(=O)O); 176.90 (*C*(=O)O); 170.67 (*C*(=O)O (last repeat unit)); 132.43 (*m*-CH (phenyl end-group)); 128.25 (*p*-CH (phenyl end-group)); 126.62 (*o*-CH (phenyl end-group)); 79.26 (w, *C*≡CH (end-group)); 71.74 (C≡CH (end-group)); 58.89 (*C*CH$_3$ (last repeat unit)); 54.32 (*C*H$_2$ (backbone)); 52.78 (OCH$_3$ (last repeat unit)); 51.75 (OCH$_3$); 44.78 (*C*CH$_3$ (backbone)); 44.42 (*C*CH$_3$ (backbone)); 34.26 (C(=O)CH$_2$CH$_2$ (end-group)); 31.50 (C(=O)CH$_2$ (end-group)); 29.19 (CH$_2$-C≡CH (end-group)); 26.85 (CCH$_3$ (last repeat unit)); 18.62 (CCH$_3$ (backbone)); 16.22 (CCH$_3$ (backbone)); 14.05 (C(*C*H$_3$)CN (end-group)).

P2: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.83 (m, 2H, *o*-C*H* (phenyl end-group)); 7.50 (m, 1H, *p*-C*H* (phenyl end-group)); 7.33 (m, 2H, *m*-C*H* (phenyl end-group)); 4.07 (br, 2n+2H, C(=O)OC*H*$_2$CH$_2$ and C*H*$_2$-C≡CH (end-group)); 3.66 (br, 2nH, C(=O)OCH$_2$C*H*$_2$); 3.60 (br, 2nH, C*H*$_2$CH$_2$OCH$_3$); 3.54 (br, 2nH, CH$_2$C*H*$_2$OCH$_3$); 3.37 (br, 3nH, CH$_2$CH$_2$OC*H*$_3$); 2.33 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.26 (w, 1H, C≡C*H* (end-group)); 2.10 – 1.55 (br, 2nH, C*H*$_2$ (backbone)); 1.50 – 1.15 (w, 5H, C(=O)CH$_2$C*H*$_2$(C*H*$_3$) (end-group)); 1.10 – 0.70 (br, 3nH, CC*H*$_3$ (backbone)).

P3: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.83 (m, 2H, *o*-C*H* (phenyl end-group)); 7.48 (m, 1H, *p*-C*H* (phenyl end-group)); 7.34 (m, 2H, *m*-C*H* (phenyl end-group)); 4.02 (w, 2H, C*H*$_2$-C≡CH (end-group)); 2.34 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.22 (w, 1H, C≡C*H* (end-group)); 2.10 – 1.55 (br, 2nH, C*H*$_2$ (backbone)); 1.50 – 1.15 (br, 9n+5H, C(C*H*$_3$)$_3$ and C(=O)CH$_2$C*H*$_2$(C*H*$_3$) (end-group)); 1.15 – 0.70 (br, 3nH, CC*H*$_3$ (backbone)).

P4: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.84 (m, 2H, *o*-C*H* (phenyl end-group)); 7.49 (m, 1H, *p*-C*H* (phenyl end-group)); 7.33 (m, 2H, *m*-C*H* (phenyl end-group)); 4.07 (br, 2nH, C(=O)OC*H*$_2$CH$_2$); 3.65 (br, 2nH, C(=O)OCH$_2$C*H*$_2$); 3.59 (br, 2nH, C*H*$_2$CH$_2$OCH$_3$); 3.53 (br, 2nH, CH$_2$C*H*$_2$OCH$_3$); 3.36 (br, 3nH, CH$_2$CH$_2$OC*H*$_3$); 2.83 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.10 – 1.63 (br, 2nH, C*H*$_2$ (backbone)); 1.50 – 1.15 (w, 5H, C(=O)CH$_2$C*H*$_2$(C*H*$_3$) (end-group)); 1.12 – 0.70 (br, 3nH, CC*H*$_3$ (backbone)); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -153.09 (m, 2F, *o*-C*F*); -158.09 (m, 1F, *p*-C*F*); -162.56 (m, 2F, *m*-C*F*).

P5: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.82 (m, 2H, *o*-C*H* (phenyl end-group)); 7.48 (m, 1H, *p*-C*H* (phenyl end-group)); 7.33 (m, 2H, *m*-C*H* (phenyl end-group)); 2.86 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.20 – 1.70 (br, 2nH, C*H*$_2$ (backbone)); 1.50 – 1.22 (br, 9n+5H, C(C*H*$_3$)$_3$ and C(=O)CH$_2$C*H*$_2$(C*H*$_3$) (end-group)); 1.22 – 0.70 (br, 3nH, CC*H*$_3$ (backbone)). ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.52 (m, 2F, *o*-C*F*); -157.57 (m, 1F, *p*-C*F*); -161.99 (m, 2F, *m*-C*F*).

General procedure for the radical substitution of the dithioester end-group with AIBN All the radical substitution reactions at the ω-end-group followed the same general procedure. As an example, the radical substitution at PMMA (P1) with an alkyne group at the α-end-group is described: PMMA (395 mg, 0.17 mmol) and AIBN (618 mg, 3.8 mmol) were dissolved in freshly distilled dioxane (37 mL) in a 100 mL round bottom flask. The pink solution was stirred for 3 h in a preheated oil bath at 80°C under inert gas. After reduction of the solution to roughly a tenth of its volume by evaporation, the product is precipitated from a cool mixture of diethyl ether and hexane (1:1) three times and finally dried in vacuum. The conversion yielded 339 mg (76%) of a white powder (P1b), and the stability of the alkyne group to this reaction was verified via NMR spectroscopy and MALDI TOF MS. P1: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.05 (w, 2H, CH_2-C≡CH (end-group)); 3.58 (br, 3nH, OCH_3); 2.35 (w, 2H, C(=O)CH_2 (end-group)); 2.26 (w, 1H, C≡CH (end-group)); 2.10 – 1.60 (br, 2nH, CH_2 (backbone)); 1.50 – 1.10 (w, 11H, C(=O)CH_2CH_2(CH_3) and C(CH_3)CN (both end-groups)); 1.08 – 0.50 (br, 3nH, CCH_3 (backbone)); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 178.07 (*C*(=O)O); 177.77 (*C*(=O)O); 176.84 (*C*(=O)O); 79.29 (w, *C*≡CH (end-group)); 71.77 (C≡*C*H (end-group)); 54.32 (*C*H_2 (backbone)); 51.76 (O*C*H_3); 44.78 (*C*CH_3 (backbone)); 44.42 (*C*CH_3 (backbone)); 34.16 (C(=O)*C*H_2*C*H_2 (end-group)); 31.53 (C(=O)*C*H_2 (end-group)); 29.22 (*C*H_2-C≡CH (end-group)); 18.63 (C*C*H_3 (backbone)); 16.22 (C*C*H_3 (backbone)); 14.06 (C(*C*H_3)CN (both end-groups)); GPC (THF): M_n = 2600 g/mol, M_w = 3200 g/mol, PDI = 1.21. MALDI TOF MS (*m*/*z*): calcd. for $C_9H_{11}N_2O(C_5H_8O_2)_{27}C_4H_6N$: 2934.8; found: 3009.0 $[M -H +2K]^+$; 2995.5 $[M -H +Na +K]^+$; 2979.7 $[M -H +2Na]^+$.

The same experimental procedure could be applied on PDEGMEMA (P2/4) and P(*t*-BuMA) (P3/5) with either a pentafluorophenyl ester or an alkyne group as α-end-group. Depending on the polymer backbone, different precipitants were used, namely a cold mixture of diethyl ether and hexane (1:1) for PDEGMEMA and a mixture of methanol and deionized water (1:1) for P(*t*-BuMA).

^{1}H NMR (300 MHz, $CDCl_3$): The ^{1}H NMR spectra for these four polymers exhibited the same peaks as the spectra of the four corresponding polymers before conversion with AIBN, except that the three multiplets of the aromatic protons between 7.9 ppm and 7.3 ppm disappeared and the signal at 1.24 ppm, representing the methyl groups bond to the same carbon atom as the nitrile group, increased in intensity upon substitution of the dithioester end-group by an isobutyronitrile group. ^{19}F NMR (377 MHz, $CDCl_3$): The ^{19}F NMR spectra of P4b and P5b did not show any changes in comparison to P4 and P5, respectively. GPC results and yields obtained are listed in the following: P2b: GPC (THF): M_n = 3900 g/mol, M_w = 4700 g/mol, PDI = 1.21; yield: 93%. P3b: GPC (THF): M_n = 3800 g/mol, M_w = 4500 g/mol, PDI = 1.18; yield: 86%. P4b: GPC (THF): M_n = 2800 g/mol, M_w = 3200 g/mol, PDI = 1.15; yield: 84%. P5b: GPC (THF): M_n = 2300 g/mol, M_w = 2700 g/mol, PDI = 1.16; yield: 63%.

Conversion of the activated ester end-group of PDEGMEMA into an alkyne end-group A degassed solution of PDEGMEMA (P4b) (510 mg, 0.18 mmol) with a pentafluorophenyl ester α-end-group and an isobutyronitrile ω-end-group, propargylamine (63 μL, 0.91 mmol), and triethylamine (78 μL, 0.46 mmol) in dry THF (10 mL) was stirred at 35°C for 3 h and then at room temperature over night. The volume of the solution was reduced by evaporation of ¾ of the solvent in order to allow for precipitation of the polymer in a cold mixture of diethyl ether and hexane (1:1). The yellowish, viscous product was obtained after two precipitations in this solvent mixture, a third precipitation in cold hexane, and drying in vacuum (yield: 92%). P4c: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.07 (br, 2n+2H, C(=O)OCH_2CH$_2$ and CH_2-C≡CH (end-group)); 3.66 (br, 2nH, C(=O)OCH$_2$CH_2); 3.60 (br, 2nH, CH_2CH$_2$OCH$_3$); 3.54 (br, 2nH, CH$_2$CH_2OCH$_3$); 3.37 (br, 3nH, CH$_2$CH$_2$OCH_3); 2.32 (w, 2H, C(=O)CH_2 (end-group)); 2.24 (w, 1H, C≡CH (end-group)); 2.15 – 1.55 (br, 2nH, CH_2 (backbone)); 1.55 – 1.15 (w, 11H, C(=O)CH$_2$CH_2(CH_3) and C(CH_3)CN (both end-groups)); 1.15 – 0.65 (br, 3nH, CCH_3 (backbone)). ^{19}F NMR (376 MHz, $CDCl_3$): no residual ^{19}F signal. GPC (THF): M_n = 2700 g/mol, M_w = 3200 g/mol, PDI = 1.16.

Conversion of the activated ester end-group of PDEGMEMA into an azide end-group The conversion of the pentafluorophenyl ester end-group of PDEGMEMA (P4b) with 1-azido-3-aminopropane, which was synthesized following a procedure described elsewhere,[25] was conducted following the same general procedure as the conversion with propargylamine, but the reaction was only stirred for 2 h at 35°C and then 15 h at room temperature. For the three precipitations, a mixture of diethyl ether and hexane (1:5) was used yielding a viscous, yellowish product (70%). P4d: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 4.07 (br, 2nH, C(=O)OCH_2CH_2); 3.66 (br, 2nH, C(=O)OCH_2CH_2); 3.60 (br, 2nH, CH_2CH_2OCH_3); 3.54 (br, 2nH, CH_2CH_2OCH_3); 3.37 (br, 3n+4H, CH_2CH_2OCH_3 and N_3CH_2CH_2CH_2 (end-group)); 2.31 (w, 2H, C(=O)CH_2 (end-group)); 2.15 – 1.55 (br, 2n+2H, CH_2 (backbone) and N_3CH_2CH_2 (end-group)); 1.55 – 1.15 (w, 11H, C(=O)CH_2CH_2(CH_3) and C(CH_3)CN (both end-groups)); 1.15 – 0.65 (br, 3nH, CCH_3 (backbone)). ^{19}F NMR (376 MHz, $CDCl_3$): no residual ^{19}F signal. FT-IR (ATR, cm^{-1}): 2938, 2883, and 2820 (br, CH_2, CH_3, O-CH_3), 2257 (w, C≡N), 2097 (w, N_3), 1726 (s, C=O), 1472, 1452, 1246, 1109 (br, C-O-C and C(=O)-O-C), 1030, 862, 748 (CH_2). GPC (THF): M_n = 2800 g/mol, M_w = 3300 g/mol, PDI = 1.15.

Conversion of the activated ester end-group of P(*t*-BuMA) into an azide end-group The pentafluorophenyl ester end-group of P(*t*-BuMA) (P5b) with an isobutyronitrile ω-end-group was transformed into an azide end-group according to the same procedure described for PDEGMEMA. Briefly, P(*t*-BuMA) (520 mg, 0.16 mmol), 1-azido-3-aminopropane (79 mg, 0.79 mmol), and triethylamine (68 µL, 0.39 mmol) were dissolved in dry THF (10 mL). This degassed solution was stirred at 35°C for 2 h and then at room temperature for 15 h. After evaporation of most of the solvent, the polymer was precipitated twice in a mixture of methanol and deionized water (1:1) and dried in vacuum, yielding 309 mg of a white powder (59%). P5d: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 3.43 – 3.25 (w, 4H, N_3CH_2CH_2CH_2 (end-group)); 2.32 (w, 2H, C(=O)CH_2 (end-group)); 2.12 – 1.70 (br, 2n+2H, CH_2 (backbone) and N_3CH_2CH_2 (end-group)); 1.50 – 1.22 (br, 9n+11H, n C(CH_3)$_3$ and C(=O)CH_2CH_2(CH_3) and C(CH_3)CN (end-groups)); 1.22 – 0.70 (br, 3nH, CCH_3 (backbone)). ^{19}F NMR (376 MHz,

$CDCl_3$): no residual ^{19}F signal. FT-IR (ATR, cm^{-1}): 2979 and 2937 (br, CH_3, CH_2), 2257 (w, C≡N), 2098 (w, N_3), 1717 (s, C=O), 1457, 1393, 1367, 1275, 1250, 1137 (C(=O)-O-C), 911, 846, 730 (CH_2), 648. GPC (THF): M_n = 2300 g/mol, M_w = 2700 g/mol, PDI = 1.17.

Synthesis of P(*t*-BuMA)-*block*-PDEGMEMA via copper(I)-catalyzed azide-alkyne-cycloaddition (CuAAC) P(*t*-BuMA) (P5d) with an α-azide-end-group (53 mg, $2.3*10^{-5}$ mol), PDEGMEMA (P2b) with an α-alkyne-end-group (134 mg, $3.4*10^{-5}$ mol) and CuBr (7.2 mg, $5.0*10^{-5}$ mol) were mixed in a Schlenk tube in dry THF (1.2 mL). This mixture was degassed by two freeze-pump-thaw cycles and the flask was refilled with nitrogen. In a separate Schlenk tube, a solution of *N,N,N′,N′′,N′′*-pentamethyldiethylenetriamine, PMDETA (9.1 mg, 11 µL, $5.3*10^{-5}$ mol) in dry THF (2 mL) was degassed by the same procedure and then transferred to the first solution. The complete reaction mixture was stirred under nitrogen for 24 h in a 40°C hot oil bath and turned green and turbid. After filtration over silica gel, which was washed with THF, the solvent was evaporated from the clear, yellowish solution and the raw product was dried in vacuum to allow for characterization prior to further purification. Afterwards, it was dissolved in THF (1 mL) and precipitated in cold Milli-Q water (20 mL). After drying in vacuum, a colorless, viscous polymer was obtained (yield: 65%).

^1H NMR (300 MHz, $CDCl_3$): δ (ppm) = 7.62 (w, 2H, N*H*); 4.49 (w, 2H, NHC(=O)CH_2 (conjugated end)); 4.08 (br, 2nH, C(=O)OC$H_2$$CH_2$); 3.66 (br, 2n+2H, C(=O)OCH_2CH_2 and C$H_2$$CH_2$$CH_2$NHC(=O) (conjugated end)); 3.60 (br, 2nH, C$H_2$$CH_2OCH_3$); 3.53 (br, 2n+2H, CH_2CH_2OCH_3 and $CH_2$$CH_2CH_2$NHC(=O) (conjugated end)); 3.37 (br, 3n, br, $CH_2$$CH_2OCH_3$); 2.31 (w, 4H, C(=O)C$H_2$ (conjugated ends)), 2.20 – 1.55 (br, 2x (2nH) + 2H, CH_2 (backbones) and CH_2C$H_2$$CH_2$NHC(=O) (end-group)); 1.40 (br, 9nH, C(CH_3)$_3$); 1.55 – 0.45 (br, 2x (3n+11H), CCH_3 (backbones) and C(=O)CH_2CH_2(CH_3) (conjugated ends) and C(CH_3)CN (both end-groups)). FT-IR (ATR, cm^{-1}): 2982, 2973, and 2862 (br, CH_2, CH_3, O-CH_3), 1727 (s, C=O), 1457, 1393, 1367, 1248, 1138 (C(=O)-O-

C), 1110 (C-O-C and C(=O)-O-C), 1095, 1068, 911, 848. GPC (THF): M_n = 6200 g/mol, M_w = 7800 g/mol, PDI = 1.26.

Conclusion

The versatility of the PFP-CTA for the synthesis of polymers with different functional α-end-groups was demonstrated. Via aminolysis of the activated ester in the CTA, another functional CTA with an alkyne group could easily be obtained, which was then used for the controlled polymerization of different methacrylate monomers. The successful incorporation of the alkyne group into the polymethacrylates could be verified via ^{1}H and ^{13}C NMR spectroscopy as well as MALDI TOF MS, which also showed the stability of this alkyne group toward the excessive treatment with AIBN, which was applied to convert the dithioester ω-end-group into the more stable isobutyronitrile group. As a second synthetic pathway toward such end-group functionalized polymethacrylates, the PFP-CTA was used directly for RAFT polymerizations and then converted into different functional α-end-groups via aminolysis after the polymerization. This method also provided an alkyne-functionalized polymer and further allowed for the synthesis of polymers with a more labile end-group, namely an azide end-group. The high functionalization efficiency of this method could be demonstrated in a polymer-to-polymer coupling reaction via CuAAC, in which the azide-functionalized building block was consumed almost completely and the diblock copolymer P(*t*-BuMA)-*block*-PDEGMEMA was obtained and purified successfully from residual PDEGMEMA, which was used in excess. In summary, the PFP-CTA enables the synthesis of well-defined polymers with functional α-end-groups via two easy but very efficient methods, which can be used depending on the nature of the desired functional end-group, and thus allows for high combinatory flexibility in polymer synthesis via RAFT polymerization facilitating the search for efficient polymer conjugation strategies.

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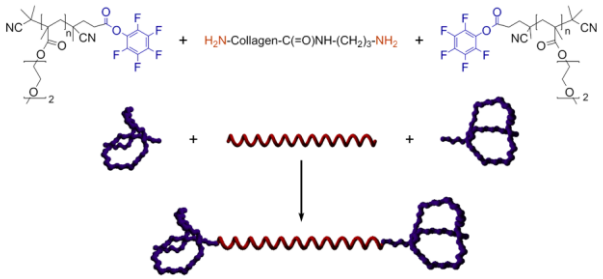
4.3 Thermo-responsive Polymer-Peptide-Conjugates on the Basis of a Collagen-like Peptide

4.3.1 A Versatile Grafting-to Approach for the Bioconjugation of Polymers to Collagen-like Peptides Using an Activated Ester Chain Transfer Agent

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



Abstract

The stimuli-responsive poly(diethylene glycol methyl ether methacrylate) was synthesized via RAFT polymerization using a functional chain transfer agent, which resulted in an activated ester end-group. This well-defined polymer could be conjugated onto the two amine-functionalized chain ends of a collagen-like peptide, which contained an interior Boc-protected lysine. The GPC elugram of the product showed neither diblock formation nor residual homopolymers, indicating the quantitative reaction of the peptide at the chain end of the polymer. Moreover, after deprotection of the peptide, observation by CD spectroscopy clearly indicated that the polymer-*b*-collagen-*b*-polymer triblock copolymer showed the triple-helical assembly characteristic of the collagen-like peptide. The triblock copolymers provide new peptide-containing macromolecules in which both block types show stimuli-responsive behavior, which should provide interesting opportunities to modulate

self-assembly behavior. This synthetic approach for the site-selective conjugation of RAFT polymers should be broadly applicable for the modification of peptides and polypeptides with well-defined synthetic polymers.

Introduction

Biohybrid materials consisting of synthetic polymers and biological moieties have gained more and more interest in the recent years.[1-10] The combination of these two material classes on the molecular scale offers not only the opportunity to overcome the limitations of the single building blocks but also the chance to design new materials that show improved or emergent properties based on the individual physicochemical and biological properties of the components. Of particular interest are block copolymers combining advantageous features of synthetic polymers, i.e. flexibility in the design of architecture and functionality,[11-14] solubility, processability, and biocompatibility as well as stimuli-responsive behavior, with advantageous features of peptides and polypeptides,[15] i.e. monodispersity and defined primary structure, controlled secondary structures, programmed assembly, and bioactivity, to yield materials that can interact with biology.[16-20] Moreover, such biohybrid polymers offer myriad opportunities to exert control over nanoscale structure; thus study of their self-assembly and stimuli-responsive behavior may increase our understanding of molecular processes in complex biological systems.[21-24]

Modern polymerization techniques, such as controlled radical polymerization methods, enable the design of well-defined synthetic polymers. The recent development in the synthesis of numerous functional initiators or chain transfer agents for these controlled radical polymerization techniques provides a versatile toolbox for the synthesis of peptide-reactive polymers with well-defined architecture.[25-31] Recently, Theato and coworkers reported the synthesis of a functional chain transfer agent (CTA) for reversible addition-fragmentation chain transfer (RAFT) polymerization containing a single activated ester end-group.[32] This CTA could be used for the controlled polymerization of a wide range of monomers

and the end-groups of the resulting polymers could easily be functionalized via conversion of the activated ester with different amines. Kiick and coworkers have designed and synthesized a novel collagen-like peptide that is capable of forming thermally stable triple-helical structures, as well as higher-order assembled structures, under mild conditions.[33,34]

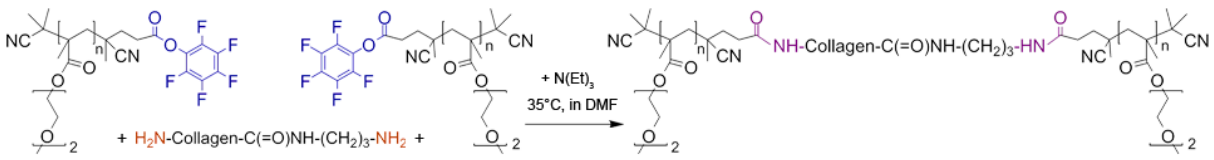
In this communication, we present the use of the RAFT CTA for the covalent conjugation of the thermally responsive polymer, poly(diethylene glycol methyl ether methacrylate) (PDEGMEMA)[35,36] to the collagen-like peptide equipped with amine groups at both the N- and C-termini. The use of these two building blocks was motivated by our interests in pre-assembly of thermally responsive triblock polymers through the biologically active collagen-like peptide domain prior to collapse of the polymer domain. After deprotection of the peptide sequence, the synthesized triblock structure shows expected assembly into collagen-like triple-helices in aqueous solution, as indicated by circular dichroic (CD) spectroscopy.

Results and Discussion

The stimuli-responsive polymer, PDEGMEMA, was synthesized via RAFT polymerization using pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) as CTA following a standard polymerization procedure as described previously.[32] The polymer with a molecular weight of M_n = 5600 g/mol and with a molecular weight distribution of M_w/M_n = 1.26 featured a reactive α-end-group as the pentafluorophenyl ester, which can be reacted with an amine-terminated peptide sequence or the amine group of a lysine residue. Hence, no post-polymerization functionalization is necessary to convert the polymer α-end-group into a reactive end-group. However, the ω-dithioester end-group resulting from the RAFT process is known to be labile toward aminolysis and would cause the loss of one equivalent of the amino-functionalized species per synthetic polymer block. To avoid the undesired loss of one equivalent of peptide per polymer chain used, the dithioester end-group was radically substituted beforehand with an isobutyronitrile group via

the conversion of the RAFT polymer with a 20-fold excess of AIBN in dioxane at 80°C for 2.5 hours following the procedure by Perrier.[37]

The collagen-like peptide was synthesized via automated Fmoc solid-phase peptide synthesis (SPPS) in *N,N*-dimethylformamide (DMF) carried out on a 2-chlorotrityl chloride resin (CLTR); the resin was pre-functionalized with 1,3-diaminopropane in order to obtain a peptide sequence with reactive amine groups at both chain ends. Selective protection of an internal lysine in the sequence was achieved by taking advantage of the higher stability of the *tert*-butyloxycarbonyl (Boc) protecting group compared to the linkage of the peptide to the CLTR resin. After the SPPS, mild cleavage of the peptide with 20% 1,1,1,3,3,3-hexafluoro-2-propanol in dichloromethane (2 hours at room temperature) from the resin was performed, yielding a fully protected collagen-like peptide[38] with a molecular weight of 4786.9 g/mol (determined by ESI-MS, m/z = 1595.5 [(M + 3H)$^{3+}$, calc: 1595.8]) after purification by RP-HPLC. The deprotected collagen-like peptide forms thermally stable triple-helices (T_m ~ 45°C) in aqueous solution as indicated by CD spectroscopy and differential scanning calorimetry,[33,34,39] indicating its promise as an assembling domain in bioactive materials; its sequence is shown in **Scheme 1**. The thermal stability of the peptide relative to the LCST of the PDEGMEMA (LCST ~ 26°C)[35,36] is relevant to the potential thermal modulation of self-assembled structures from these building blocks and is expected to facilitate further studies on the mutual effect of these two temperature dependent phenomena on each other.



Definition: H_2N-Collagen-C(=O)NH-$(CH_2)_3$-NH_2
= H_2N-GGPPGPPGPPGPRGEKGERGPRGPPGPPGPPGPCCG-C(=O)NH-$(CH_2)_3$-NH_2.

SCHEME 1: Synthesis of a hybrid triblock copolymer via activated ester chemistry from PDEGMEMA and a collagen-like peptide with protected residual groups (cysteine: Trt, glutamic acid: t-Bu, lysine: Boc, arginine: Pbf).

The two building blocks, synthetic polymer and peptide, were conjugated to form the polymer-*b*-collagen-*b*-polymer triblock copolymer (PCP) by mixing 1.5 equivalents of PDEGMEMA per primary amine group of the peptide (**Scheme 1**). The reaction was carried out in DMF at 35°C for 2 days, and 2 µL triethylamine were added. The resulting hybrid polymer was isolated by threefold precipitation into cold diethyl ether and dried in vacuum. A GPC in DMF showed one product signal that was clearly shifted toward higher molecular weight compared to either of the building blocks (**Figure 1**).

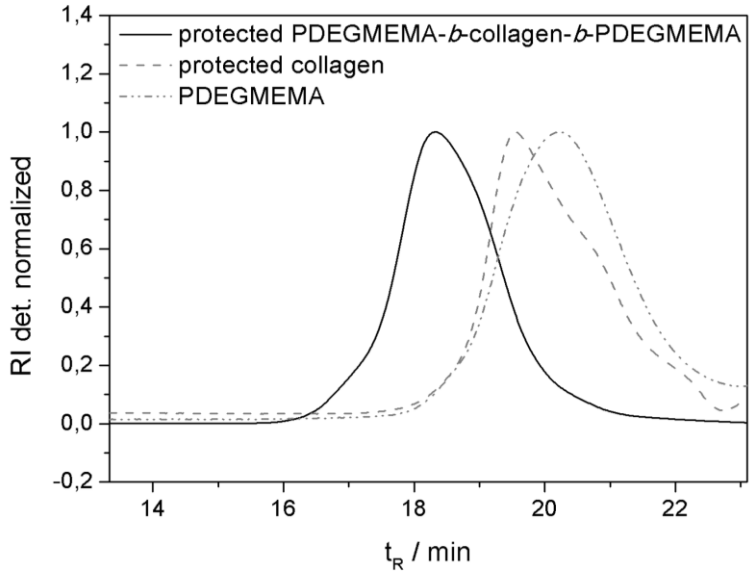


FIGURE 1: GPC elugram of the protected triblock copolymer in DMF (+ 0.01M LiCl) in comparison with the elugrams of the homopolymer and the collagen-like peptide.

The GPC trace of the peptide was plotted only for comparison, although GPC is not the ideal method to measure the monodispersity of peptides, as they tend to interact with the column material and thus cause asymmetric elugrams.[40] According to the evaluation of the GPC data via calibration with PMMA standards, the molecular weight of the triblock system was M_n = 13700 g/mol with a M_w/M_n = 1.32. The absence of a lower molecular weight shoulder indicated the absence of any unconverted peptide or homopolymer, confirming complete reaction of the peptide and successful separation of the homopolymer from the product by precipitation into cold diethyl ether.

After the successful conjugation with the polymer, the peptide was deprotected in a mixture of trifluoroacetic acid (TFA), deionized water, 1,2-ethanedithiol and triisopropylsilane (94.5:2.5:2.5:1) at room temperature (2 hours) and afterwards precipitated into a cold mixture of diethyl ether and hexane (50:50). The ester linkages in the polymer side groups were found to be stable under these conditions (see **Figure 5 and 6**). Retention of the PCP triblock structure after deprotection was verified via sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE, **Figure 2**).

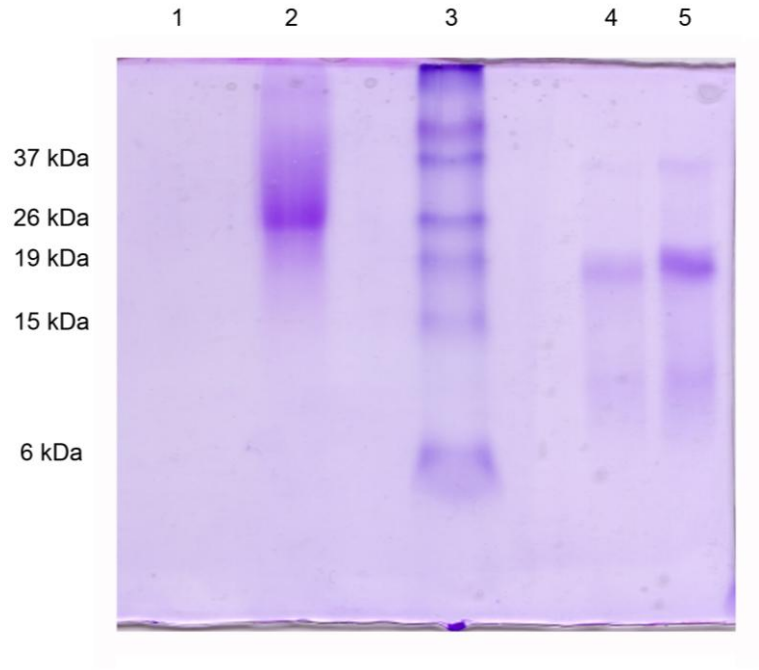


FIGURE 2: SDS PAGE: Lane 1 contains the homopolymer (not stained), lane 2 the deprotected triblock copolymer PDEGMEMA-b-collagen-b-PDEGMEMA, lane 3 a protein ladder, lane 4 a blend of homopolymer and peptide (only the peptide band is visible), lane 5 only the collagen-like peptide. The samples were run on a 14% gel and the bands were visualized via treatment with Coomassie Blue.

The gel clearly showed that the smeared band for the deprotected PCP (lane 2) is distinct from a simple blend of polymer and peptide (lane 4; multiple bands arise from the presence of both unfolded monomer and folded triple-helix in the peptide sample (as illustrated in lane 5)); the data also illustrate that the product did not contain any non-conjugated peptide and that PCP exhibits a higher molecular weight than the peptide itself.[41] The triblock structure was also indicated by ^{1}H NMR. The

spectrum of the deprotected hybrid polymer (**Figure 3a**) shows clearly the strong signals of the two polymer blocks as well as signals characteristic of the peptide block, i.e. some of the signals representing the protons at the α-carbon atoms between 5 and 4 ppm and the signals of the protons in the peptide bond (9.00 – 6.37 ppm) (for comparison with the respective building blocks see **Figure 3b** and **3c**). The sum of the integrals of the latter (36 protons per molecule) were compared to the integral of the signal for the methyl group in the polymer backbone (~164 protons in the triblock copolymer) between 1.04 and 0.52 ppm, and this comparison showed that the product contained at least 86% of the triblock copolymer and not more than 14% of the diblock copolymer was formed as byproduct.

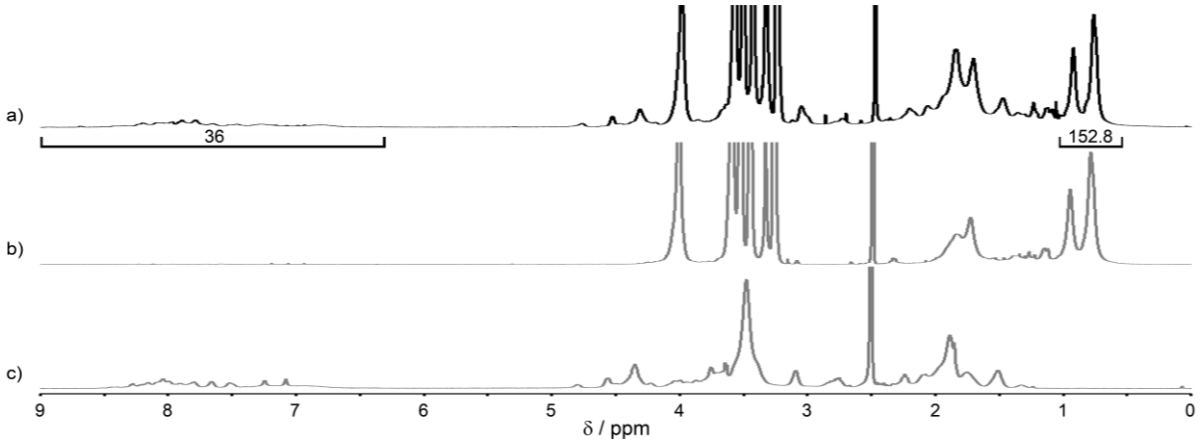


FIGURE 3: ^{1}H NMR spectra of a) deprotected PDEGMEMA-b-collagen-b-PDEGMEMA (600 MHz), b) PDEGMEMA (400 MHz) and c) the deprotected peptide (400 MHz) in d_6-DMSO.

Thus, the SDS-PAGE and ^{1}H NMR data confirm the formation of the PCP as suggested by GPC. Of functional interest, the deprotected PCP exhibits an LCST of ~38°C (onset) in water, which is higher than that of the pure homopolymer, as expected.

A 137 μM solution (concentration determined by amino acid analysis) of the deprotected PCP in phosphate buffered saline (PBS, pH = 7.4) was incubated at 4°C overnight in order to allow the peptide to form the collagen triple-helical structure. This solution was analyzed via CD spectroscopy to evaluate triple-helix formation of the peptide block under these conditions (**Figure 4a**). The CD spectrum at 5°C

featured the typical maximum of a collagen triple-helix centered at 225 nm and a minimum at 202 nm.[39,42] The CD spectrum of pure PDEGMEMA was also measured and, as expected, did not show any CD activity. Hence, these results suggest that the collagen-like peptide sequence successfully promoted the self-organization of PCP into assemblies containing triple-helices. Further, the thermal denaturation of PCP was monitored via change in mean residue ellipticity ($[\theta]_{MRE}$) at 202 and 225 nm (**Figure 4b**). While the non-functionalized collagen-like peptide showed a standard sigmoidal unfolding curve,[43] in the case of PCP, the changes in $[\theta]_{MRE}$ values at 202 and 225 nm indicated a more gradual and complicated unfolding with two potential transitions, suggesting multistate non cooperative transition behavior and a dual responsiveness caused by convolution of the thermally responsive behavior of polymer blocks and the unfolding of the collagen block.

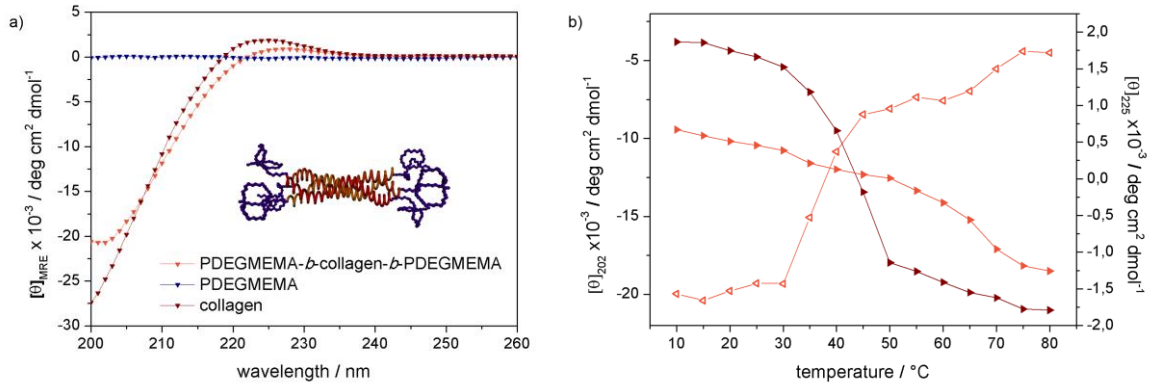


FIGURE 4: a) CD spectra of the deprotected triblock copolymer PDEGMEMA-b-collagen-b-PDEGMEMA, the homopolymer and the collagen-like peptide in PBS at 5°C, b) thermal denaturation curves of the deprotected triblock copolymer (orange hollow triangles at 202 nm, orange filled triangles at 225 nm) and the collagen-like peptide (red triangles at 225 nm) in PBS measured via CD spectroscopy.

This self-assembly behavior, the delayed thermal denaturation of the triple-helix in the presence of the polymer, and the potential of this hybrid material to form nanometer-scale structures are extremely promising given the organization of the isolated peptide domain into nano- and micro-scale structures as suggested by electron microscopy.[34] Further, the stimuli-responsive character of the involved

polymer blocks offers intriguing possibilities to modulate the behavior of the biohybrid polymer[44] and is currently under investigation.

Experimental

Materials. All chemicals and solvents were commercially available and used as received unless mentioned otherwise. 2,2′-Azobisisobutyronitrile (AIBN) was recrystallized from diethyl ether. Diethylene glycol monomethylether methacrylate (DEGMEMA) was purified by distillation in vacuum. Dioxane and tetrahydrofurane (THF) for the polymerization, the conversion with AIBN, and dissolving the polymer during purification via precipitation were distilled from sodium / potassium.

Instrumentation. ^{1}H-NMR spectra were recorded either on a Bruker Avance II 400 FT-NMR spectrometer or on a Bruker Avance 600 FT-NMR spectrometer working at 400 and 600 MHz, respectively. ^{19}F-NMR spectra were recorded on a Bruker 376.5 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. For GPC in *N,N*-dimethylformamide containing 0.01 M LiCl a Polymer Laboratories PL-GPC50 with two PLGel 5 μm mixed-D columns (300 x 7.5 mm), a PLGel 5 μm mixed-D guard column (50 x 7.5 mm), and a Knauer RI detector was used at 50°C and a flow rate of 1 mL/min. The molecular weights and polydispersity indices were calculated using a calibration curve from poly(methyl methacrylate) standards. GPC in THF was performed on an instrument consisting of a Waters 717 plus autosampler, a TSP Spectra Series P 100 pump, and a set of three MZ-Gel SD plus columns with 100, 1000, and 10000 Å porosity. The eluent was used at room temperature and a flow rate of 1 mL/min. The specific refractive index increment (dn/dc) was measured on an Optilab DSP interferometric refractometer (RI detector).
Reversed-phase high performance liquid chromatography (RP-HPCL) was performed on a system consisting of a Waters 717 plus autosampler, a Waters 600

controller, a Waters Symmetry 300 C18 column (5 μm, 19 x 150 mm), a Waters Fraction Collector III, and a Waters 2996 Photodiode Detector connected to the software Empower Pro build 1154.

For the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), samples were dissolved in 10 mM phosphate buffered saline (PBS, 150 mM NaCl, pH 7.4), run on a 14% gel at 5°C and visualized via treatment with Coomassie Blue. For comparison, a protein ladder purchased from Invitrogen was used. However, it should be mentioned that the peptide and the hybrid bands do not correlate directly to the molecular weight ladder. It has been commonly observed previously that collagen-like peptides with high proline content migrate slower in SDS-PAGE than globular proteins which are used in the commercial protein ladder because of the higher rigidity of the collagen chain even in the denaturated state.[41]

The cloud point was determined by optical transmittance of a laser light beam ($\lambda = 632$ nm) through a 2 mm sample cell at temperatures from 10° to 60°C at a heating rate of 0.2°C/min using a Jasco V-630 spectrophotometer equipped with a Jasco ETC-717 Peletier element. The sample concentration in water was 2.2 mg/mL.

Circular dichroic spectroscopy was performed on a Jasco J-810 spectropolarimeter equipped with a Jasco PTC-424S Peltier temperature controller and a 1 mm quartz cell. The spectra were recorded from 260 to 200 nm at a rate of 50 nm/min at 5°C. The ellipticity values, Θ in mdeg, were converted to mean residue ellipticity values, $[\Theta]_{MRE}$ in deg cm^2 dmol^{-1}, according to the following formula: $[\Theta]_{MRE} = (\Theta * m) / (c * l * n_f)$, where m is the molecular weight in g/mol, c is concentration in mg/mL (determined by amino acid analysis of the hybrid solution), l is the path length of the cuvette in mm, and n_f is the number of amino acid residues present in the peptide. For the denaturation curves, the CD spectra were measured at different temperatures and the minimum (at 202 nm) and maximum (at 225 nm) of $[\Theta]_{MRE}$ were plotted versus temperature.

Synthesis of PDEGMEMA via RAFT Polymerization.[32] In a Schlenk flask equipped with stir bar, 1.5 g (7.97 mmol) DEGMEMA, 177 mg (0.40 mmol) pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate), 6.5 mg (0.04 mmol) AIBN and 3 mL of freshly distilled dioxane were combined. The solution was degassed by three freeze-pump-thaw cycles and the flask was refilled with argon. The polymerization was performed in a preheated and stirred oil bath set to 70°C for 20 hours. The polymer was then precipitated three times into cold hexane and dried in vacuum. The reaction yielded 1.23 g of a dark red, viscous polymer (82 %). ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.86 (m, 2H); 7.50 (m, 1H); 7.34 (m, 2H); 4.08 (br, 2H); 3.66 (br, 2H); 3.60 (br, 2H); 3.54 (br, 2H); 3.37 (br, 3H); 2.40 (w, 2H), 2.10 – 1.70 (br, 2H), 1.50 – 1.10 (w, 5H); 1.10 – 0.75 (br, 3H); ^{19}F NMR ($CDCl_3$): δ (ppm) = -153.09 (m, 2F); -158.09 (m, 1F); -162.56 (m, 2F); GPC (THF): M_n = 5600 g/mol, M_w = 7000 g/mol, PDI 1.26.

Conversion of the RAFT end-group with AIBN.[37] 1.2 g (0.21 mmol) PDEGMEMA, containing the dithioester end-group from the RAFT polymerization, and 20 equivalents of AIBN (0.72 g, 4.4 mmol) were dissolved in 45 mL dry dioxane. The mixture was heated to 80°C for 2.5 hours and the resulting yellowish, viscous polymer was precipitated three times into a cold mixture of hexane and diethyl ether (50:50) and dried in vacuum. A ^{1}H NMR spectrum showed no remaining residues of AIBN, the replacement of the phenyldithioester (disappearance of the aromatic signals) and the reaction was almost quantitative. A ^{19}F NMR showed the three characteristic peaks of the pentafluorophenylester end-group. ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.08 (br, 2H); 3.66 (br, 2H); 3.60 (br, 2H); 3.54 (br, 2H); 3.37 (br, 3H); 2.40 (w, 2H); 2.10 – 1.70 (br, 2H); 1.50 – 1.10 (w, 11H); 1.10 – 0.75 (br, 3H); ^{1}H NMR (DMSO): 4.01 (br, 2H); 3.59 (br, 2H); 3.52 (br, 2H); 3.45 (br, 2H); 3.26 (br, 3H); 2.33 (w, 2H); 2.10 – 1.55 (br, 2H); 1.55 – 1.05 (w, 11H); 1.04 – 0.52 (br, 3H); ^{19}F NMR ($CDCl_3$): δ (ppm) = -153.09 (m, 2F); -158.09 (m, 1F); -162.56 (m, 2F); GPC (THF): M_n = 5600 g/mol, M_w = 7000 g/mol, PDI = 1.26; GPC (DMF): M_n = 2500 g/mol, M_w = 3400 g/mol, PDI = 1.38.

Peptide Synthesis and Purification. The collagen-like peptide was synthesized using automated N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) solid-phase peptide synthesis on a Protein Technologies Inc PS3 peptide synthesizer in DMF according to the work of Kiick and coworkers[33,34] with slight modification. Briefly, the peptide sequence was built up on a 2-chlorotrityl chloride resin (CLTR) which was pre-functionalized with 1,3-diaminopropane in order to obtain a peptide with amine groups at both chain ends. Cleavage of the peptide from the resin was performed with 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in dichloromethane (2 hours at room temperature). The cleaved peptide was separated from the resin by filtration through a frit followed by extensive washing of the resin with dichloromethane. The filtrate was precipitated into cold diethyl ether and recovered by centrifugation for 20 min at 4000 rpm. The white solid was dissolved in a mixture of water and acetonitrile (50:50) and lyophilized. The dried peptide was dissolved in water and acetonitrile (50:50) again (~10 mg/mL) and purified via RP-HPLC with a mobile phase of 0.1% trifluoroacetic acid (TFA) and a linear 20-min gradient of 50 to 80% acetonitrile followed by a linear 30-min gradient of 80 to 100% acetonitrile. A typical chromatogram yielded a major peak elution at 81% acetonitrile. The collected fractions were lyophilized prior to characterization. ESI-MS: MW = 4786.9 g/mol (m/z = 1595.5 [$(M+3H)^{3+}$, calc: 1595.8]). GPC (DMF): M_n = 4900 g/mol, M_w = 6700 g/mol, PDI = 1.37.

In order to allow comparison with NMR spectrum of the deprotected triblock copolymer PCP, some peptide was deprotected under the same procedure as described below for the deprotection of PCP and analyzed via NMR spectroscopy. ^{1}H NMR (DMSO): δ (ppm) = 8.45; 8.43; 8.38; 8.28; 8.26; 8.25; 8.21; 8.18; 8.15; 8.07; 8.02; 7.98; 7.93; 7.90; 7.89; 7.86; 7.78; 7.64; 7.51; 7.23; 7.07; 4.79; 4.56; 4.49; 4.34; 4.21; 4.02; 3.98; 3.93; 3.86; 3.74; 3.70; 3.69; 3.66; 3.63; 3.62; 3.47; 3.08; 2.90 – 2.64 (m); 2.46 – 2.35 (m); 2.31; 2.23; 2.08; 2.06; 1.87; 1.84; 1.74; 1.50; 1.32; 1.22.

Synthesis of PDEGMEMA-*b*-collagen-*b*-PDEGMEMA (PCP). In order to conjugate two blocks of poly(diethylene glycol methyl ether methacrylate) (PDEGMEMA) to the protected collagen-like peptide (sequence see **Scheme 1**), a solution of 10.3 mg ($2.15*10^{-6}$ mol) freeze-dried peptide in 2 mL anhydrous dimethylformamide (DMF) is added to 36.1 mg ($6.45*10^{-6}$ mol, 3 equivalents) polymer in a glass vial equipped with a stir bar. Roughly 2 µL triethylamine (at least 0.9 µL which corresponds to one equivalent triethylamine compared with the activated ester end-group of the polymer) are added and the glass vial is capped with a septum. The almost colorless solution is placed in a preheated oil bath at 35°C and stirred for 2 days. Afterwards, the reaction solution is dropped into 15 mL cool diethyl ether for precipitation and then this suspension is centrifuged for 15 minutes at 4000 rpm and 10°C so that the ether can be separated from the yellowish viscous PCP. The product is dissolved in 0.3 mL anhydrous DMF and again precipitated into cold diethyl ether. After threefold precipitation following the described procedure, the product is dried in vacuum (yield = 33.7 mg, ~100%). GPC (DMF): M_n = 13700 g/mol, 18100 g/mol, PDI = 1.32.

Deprotection of PCP. 30 mg of PCP are mixed with the deprotection mixture which was prepared in a separate vial previously (945 µL trifluoroacetic acid (TFA), 25 µL deionized water, 25 µL 1,2-ethanedithiol and 10 µL triisopropylsilane) and stirred for 2 hours at room temperature. At the end of the reaction time, the volume of this clear solution is reduced by evaporation of the reagents with a N_2 stream and the viscous product is dissolved in 0.3 mL tetrahydrofuran and then precipitated into a cold mixture of diethyl ether and hexane (50:50). After centrifugation (4000 rpm, 20 min, 10°C), the solvents could be separated from the product which is then precipitated again in the same way and finally isolated by lyophilization out of deionized water (yield = 17.7 mg, 58%). ^{1}H NMR (DMSO): δ (ppm) = 8.69; 8.43; 8.35; 8.23; 8.20; 7.98; 7.97; 7.92; 7.89; 7.79; 7.73; 7.65; 7.52; 7.46; 7.28; 7.16; 7.08; 6.99; 6.93; 6.81; 4.77; 4.53; 4.46; 4.31; 4.19; 3.98; 3.86; 3.66; 3.64; 3.57; 3.50; 3.43; 3.24; 3.12; 3.05; 2.72; 2.58; 2.39;

2.36; 2.20; 2.06; 1.94; 1.84; 1.70; 1.47; 1.36; 1.30; 1.24; 1.14; 1.12; 1.09; 1.07; 1.06; 1.05; 1.02; 0.92; 0.76.

Investigation of the stability of the ester linkages in PDEGMEMA. In order to determine the stability of the ester linkages of PDEGMEMA under the acidic deprotection conditions, the pure homopolymer (before substitution of the dithioester RAFT end-group) is treated with the same deprotection mixture as used for PCP and under the same conditions. The polymer is then precipitated into a cold mixture of diethyl ether and hexane (20:80) two times and characterized by ^{1}H NMR and ^{13}C NMR. ^{1}H and ^{13}C NMR showed the typical polymeric signals (data not shown) without any indication for cleavage of the ester linkages in the polymer side chains. Assuming that it would be hard to see single protons or carbon atoms from carboxylic groups and that the cleaved side chain could be lost during the precipitation steps, one can still not yet exclude the cleavage of just a little percentage of ester linkages in the polymer side chains. To verify their absolute stability indirectly, the stability of the activated ester end-group is monitored via ^{19}F NMR. This end-group, namely the pentafluorophenyl ester, is supposed to be much more labile than the ester groups in the side chains of the polymer. **Figure 5** shows the ^{19}F NMR spectra of PDEGMEMA before and after the treatment with the acidic deprotection mixture ("TFA treatment"). The absence of signals representing free pentafluorophenol in the spectrum of the polymer after the treatment indicates the stability of even this more labile ester group under the acidic conditions. This indication is confirmed by adding an equimolar amount of 1,2-dibromo-4,5-difluoro-benzene relative to the polymer sample to both of the NMR samples in order to quantify the integrals of the pentafluorophenyl ester signals which turned out to be identical before and after the TFA treatment. Further, the pink color of the dithioester end-group at the other chain end did not disappear neither during the described treatment, which would happen if this end-group was cleaved.

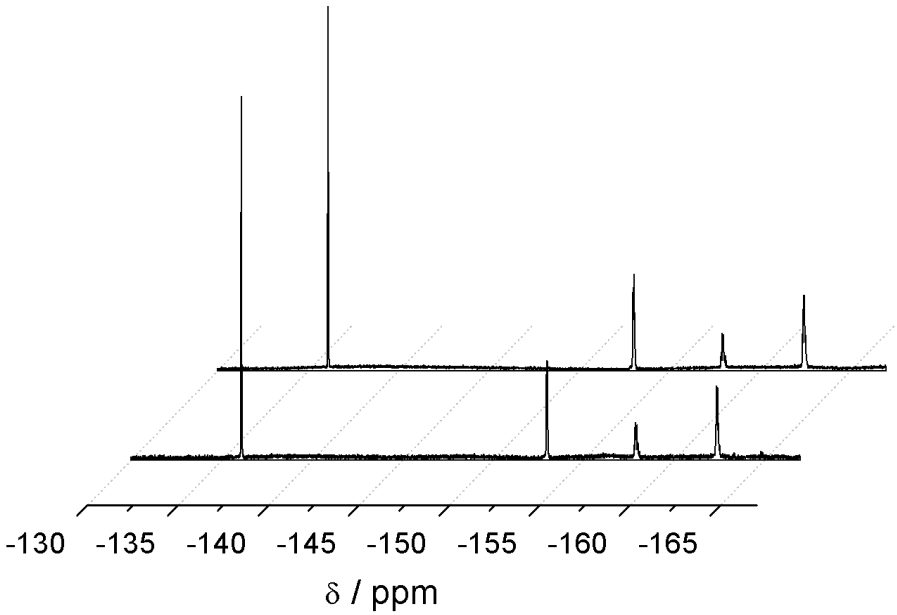


FIGURE 5: ^{19}F NMR spectra of the same PDEGMEMA sample before (back) and after (front) the treatment with the deprotection mixture, both spectra measured with an equimolar amount of 1,2-dibromo-4,5-difluoro-benzene added as a standard.

And finally, the GPC elugrams before and after TFA treatment are identical (**Figure 6**) which also confirms the stability of the polymer under the deprotection conditions.

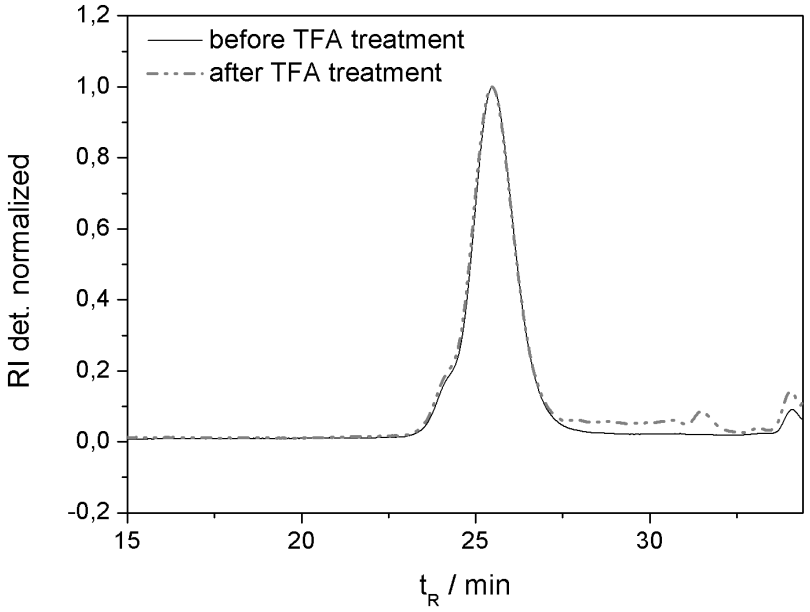


FIGURE 6: GPC elugrams of a PDEGMEMA sample before and after treatment with the deprotection mixture (mainly TFA).

Conclusion

In summary, a versatile synthetic approach for the successful bioconjugation of RAFT polymers to peptides, without post-polymerization functionalization of either of the two building blocks, was established. This approach could be applied to various peptides with addressable amine groups and to a variety of synthetic polymers amenable to synthesis by RAFT polymerization using the described functional CTA. As an example, the site-selective conjugation of a stimuli-responsive poly(methacrylate) and a collagen-like peptide containing a Boc-protected lysine was demonstrated. The resulting PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer exhibited the expected collagen triple-helical structure, suggesting opportunities to sequentially drive self-assembly behavior of the triblock via simple changes in temperature. Further studies of the self-organization of this and similar hybrid materials will follow. This synthetic approach is broadly applicable and could also be employed in the synthesis of comparable diblock copolymers or multiblock copolymers.

Acknowledgement

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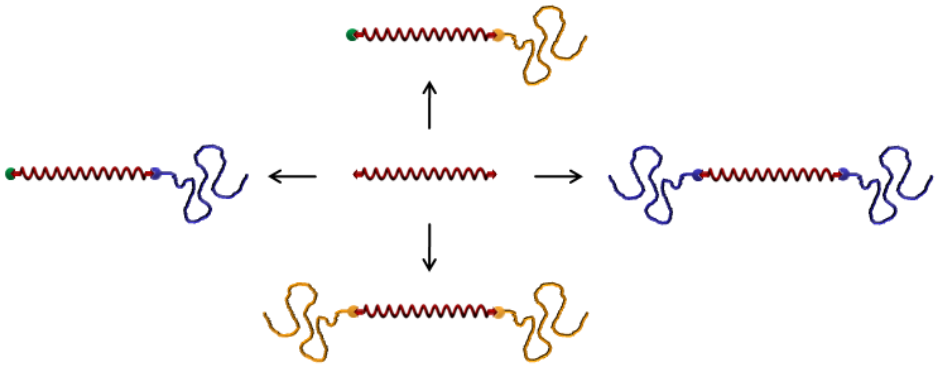
4.3.2 Hybrid Copolymers Consisting of Thermo-responsive Polymers and a Collagen-like Peptide



Introduction

Recently, a facile approach for the conjugation of polymers synthesized via RAFT polymerization to peptides has been established, which utilizes the functional CTA pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate), briefly called PFP-CTA.[1,2] This CTA is used for the synthesis of narrowly distributed polymers with a well-defined α-end-group, i.e. an activated ester, which can easily react with the amine groups of a peptide. The effectiveness of the conjugation reaction of these functional polymers to peptides via amide formation has been demonstrated on the example of a polymer-*b*-peptide-*b*-polymer triblock copolymer based on the thermo-responsive polymer poly(diethylene glycol methyl ether methacrylate) (PDEGMEMA)[3-5] and a collagen-like peptide[6,7] with two amine end-groups. This stimuli-responsive hybrid system, exhibited the characteristic signals of a triple-helical structure as known from collagen[8,9] in CD spectroscopy and showed a gradual unfolding curve of this triple-helix upon heating, suggesting a double thermo-responsive self-assembly behavior.

To verify the broader applicability of this synthetic approach, the synthesis of further polymer-peptide-conjugates via the same method is highly desirable. These novel hybrid systems should also allow for more detailed studies on the thermo-responsive

behavior of this type of collagen-based bioconjugates. First, the same peptide sequence with only one amine end-group would enable the synthesis of PDEGMEMA-*b*-collagen diblock copolymer. Second, via variation of the synthetic polymer building block, di- and triblock copolymers could be realized, in which the collapse of the synthetic building blocks is supposed to occur at higher temperatures than the unfolding of the collagen-like triple helix. For this purpose, activated ester-functionalized poly(oligoethylene glycol methyl ether methacrylate) (POEGMEMA) prepared via RAFT polymerization, which exhibits a higher LCST (~ 64°C) than PDEGMEMA (LCST ~ 26°C),[5,10] is a suitable candidate.

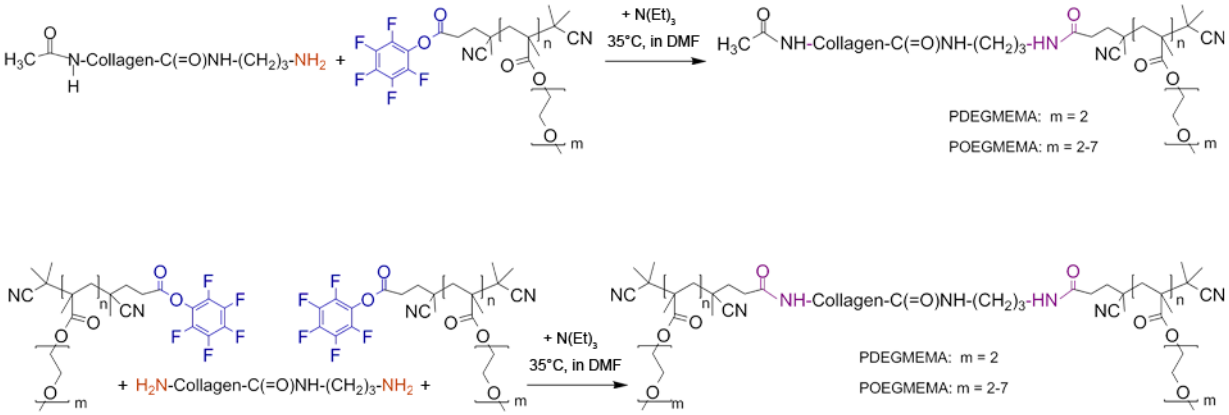
Results and Discussion

As discussed above, a polymer-*b*-peptide-*b*-polymer triblock copolymer PCP could be synthesized on the basis of a collagen-like peptide sequence with two amine end-groups, which were reacted with two synthetic polymer blocks exhibiting an activated ester as α-end-group.[1] To prevent a reaction of these functional polymers with the primary amine group of the lysine contained in the peptide sequence, it was important to use the peptide with appropriate protecting groups. Therefore, the peptide was prepared on a 2-chlorotrityl chloride resin (CLTR) via solid-phase peptide synthesis (SPPS). In contrast to the *tert*-butyloxycarbonyl (Boc) protecting group of the lysine, the 2-chlorotrityl chloride linkers are relatively labile toward hydrolysis, so that a protected peptide sequence can be cleaved from the resin, if mild cleaving conditions are chosen, i.e. 20% 1,1,1,3,3,3-hexafluoro-2-propanol in dichloromethane (2 hours at room temperature).[11] Moreover, this CLTR was prefunctionalized with 1,3-diaminopropane in order to obtain a peptide with two amine end-groups after sequential synthesis of the peptide starting at the C-terminus of the sequence. To allow a comparison of the resulting hybrid structures, the same procedure was chosen for the synthesis of the collagen-like peptide with only one amine end-group, only that the N-terminus was acetylated prior to cleavage of the peptide from the resin. Thus, only the functionalized C-terminus exhibited a free

amine group for polymer conjugation, thereby allowing the synthesis of diblock copolymers using this peptide.

For the synthesis of a PDEGMEMA-*b*-collagen diblock copolymer, the RAFT polymerization of DEGMEMA was repeated using the PFP-CTA and the same conditions and ratios of the reactants as described previously (DP = 27, M = 5500 g/mol, calculated via end-group analysis in ^{1}H NMR).[1] The α-end-group of the resulting PDEGMEMA contained the desired pentafluorophenyl ester group, as confirmed via ^{19}F NMR spectroscopy, while the dithioester at the ω-chain-end was converted into an isobutyronitrile group via radical substitution with an excess of AIBN.[1,12] Even though this end-group modification is not crucial for the conjugation reaction, it avoids the undesired loss of one equivalent of the amine-functional peptide, which could be caused by the potential aminolysis of the dithioester. The integrity of the activated ester end-group was again confirmed via ^{19}F NMR.

With the described building blocks in hand, the bioconjugation could be carried out. The activated ester end-group of the PDEGMEMA was reacted with the collagen-like peptide with one amine end-group in dry DMF (upper line of **Scheme 2**, m = 2) by stirring the solution at 35°C for 3 days.



Definition: HN-Collagen-C(=O)NH-$(CH_2)_3$-NH

= HN-GGPPGPPGPPGPRGEKGERGPRGPPGPPGPPGPCCG-C(=O)NH-$(CH_2)_3$-NH.

SCHEME 2: Synthesis of di- and triblock copolymers via activated ester chemistry from synthetic polymers and a collagen-like peptide with protected residual groups (cysteine: Trt, glutamic acid: t-Bu, lysine: Boc, arginine: Pbf).

Triethylamine was added as auxiliary base, in order to capture the leaving group of the activated esters, i.e. pentafluorophenolate. The PDEGMEMA-*b*-collagen diblock copolymer could be isolated via precipitation in cold diethyl ether, which at the same time dissolved the excess of PDEGMEMA, and was obtained in almost quantitative yield (97%).

The GPC elugram of the protected product in DMF (**Figure 6**) showed a shift to higher molecular weight in comparison to the building blocks and the complete removal of unreacted PDEGMEMA. It should be mentioned, that GPC is not necessarily an appropriate means for analysis of the polydispersity of peptides or polymer-peptide-conjugates, and that the molecular weights calculated using a calibration curve from PMMA standards do not necessarily represent the true molecular weights, because peptides can undergo interaction with the column material and thus falsify the resulting elugrams.[13] Here, the GPC elugram of the peptide was only plotted for comparison with the product. For determination of the efficiency of the conjugation and confirmation of the diblock structure, the NMR spectrum of the deprotected copolymer will be discussed below.

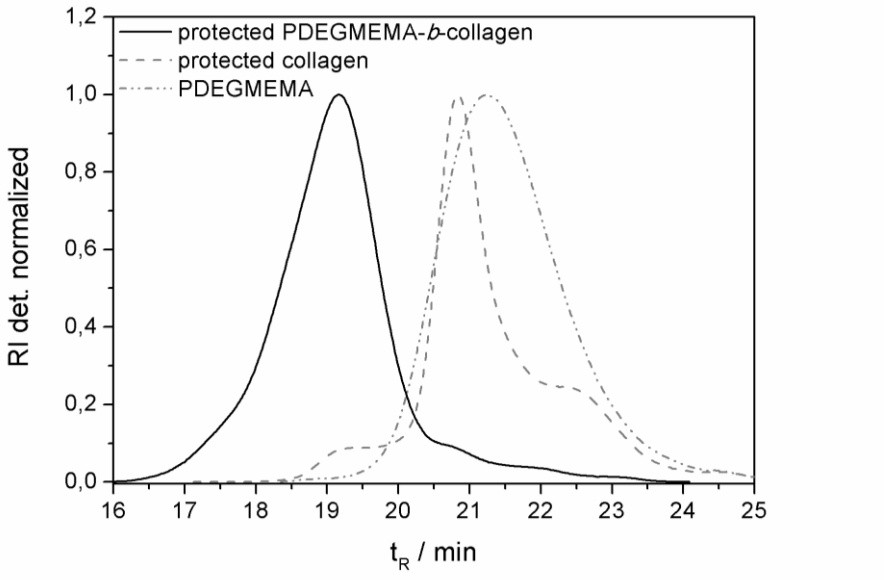


FIGURE 6: GPC elugram of the protected diblock copolymer in DMF (+ 0.25M LiBr) in comparison with the elugrams of the homopolymer and the collagen-like peptide.

The deprotection of the PDEGMEMA-*b*-collagen diblock copolymer was accomplished using a mixture of trifluoroacetic acid (TFA), deionized water, triisopropylsilane, and 1,2-ethanedithiol at room temperature. The stability of the PDEGMEMA block to this treatment was already demonstrated earlier.[1] The ^{1}H NMR spectrum of this diblock copolymer was analyzed analog to the one of the triblock copolymer described in chapter 4.3.1 (**Figure 2**): The sum of the integrals of the signals representing the 36 protons in the peptide bonds (9.37 – 5.80 ppm) is compared to the integral of the signal for the methyl group in the polymer backbone (in theory: ~82 protons in the diblock copolymer, found: integral of 82.01) between 1.03 and 0.39 ppm. That way, the ratio of the peptide block to the PDEGMEMA block was calculated to be 1:1. The good agreement of the values of the integrals found in the NMR spectrum with the calculated number of protons confirms the successful conversion of the collagen-like peptide to a polymer-*b*-peptide diblock copolymer.

In order to obtain similar di- and triblock copolymers with an LCST above the melting temperature of the collagen-like triple helix for comparison with the PDEGMEMA-based hybrid systems, POEGMEMA was synthesized, which shows an LCST of about 64°C as a homopolymer. This polymer consists of the same methacrylate backbone as PDEGMEMA, but its side chains contain more than two ethylene glycol units. The average molecular weight of the monomer is given as 300 g/mol by the provider (Sigma-Aldrich), and MALDI studies of POEGMEMA revealed a side chain distribution of two to seven ethylene glycol units per monomer.[14] These longer side chains turn the polymer into a more hydrophilic one than PDEGMEMA and cause the higher LCST. POEGMEMA with a pentafluorophenyl ester at the α-end-group was synthesized via RAFT polymerization using the PFP-CTA in the same molar ratio to the monomer (1:20) as in the synthesis of the PDEGMEMA described previously, so that the same degree of polymerization was obtained (DP = 27, M = 8550 g/mol, calculated via end-group analysis in ^{1}H NMR). Afterwards, the dithioester end-group was substituted radically with AIBN.

In general, the conjugation of POEGMEMA to the two collagen-like peptides with either one or two amine end-groups yielding a POEGMEMA-*b*-collagen diblock copolymer or a POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer, respectively, was performed in direct analogy to the syntheses of the PDEGMEMA-based copolymers (**Scheme 2**), only that the work-up had to be modified slightly due to different solubilities of the products. In contrast to the PDEGMEMA containing hybrid systems, the POEGMEMA-based copolymers did not precipitate completely in cold diethyl ether. Hence, the products were precipitated from the DMF solutions in a cold mixture of diethyl ether and hexane (10:1). The protected hybrid systems were analyzed via GPC in DMF and, analog to the first two examples, the elugrams showed pure higher molecular weight species indicating full conversion of the peptide and complete removal of the excess POEGMEMA homopolymer. The surprising observation, that the M_n of the diblock copolymer obtained via interpretation of the elugram using a calibration with PMMA standards was higher than the one calculated for the triblock copolymer, can probably be explained by the stronger stealth effect, which two POEGMEMA blocks have on the peptide (in the case of the triblock copolymer) in comparison to only one POEGMEMA block (in the case of the diblock copolymer).

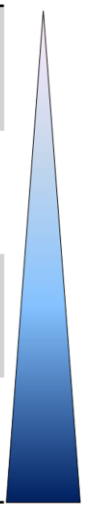
The POEGMEMA-*b*-collagen diblock copolymer and the POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer were both deprotected under the same conditions as described previously for the hydrolysis of the PDEGMEMA-*b*-collagen diblock copolymer, and could be isolated via precipitation from a cold mixture of diethyl ether and hexane (50:50) followed by lyophilization. However, the higher polarity of these hybrid systems led to lower yields (in terms of total mass gained) in the precipitation steps. Nevertheless, it was successfully shown that the synthetic scheme could be generalized to the synthesis of various polymer-peptide-conjugates.

Table 1 gives an overview of the bioconjugates obtained and the expected polarities.

TABLE 1: Overview of the bioconjugates with increasing expected hydrophilicity.

Building blocks with transition temperatures		Bioconjugates
PDEGMEMA 26°C[a]	H_2N-collagen-NH_2 45°C[b]	PDEGMEMA-*b*-collagen-*b*-PDEGMEMA
PDEGMEMA 26°C[a]	collagen-NH_2 45°C[b]	PDEGMEMA-*b*-collagen
POEGMEMA 64°C[a]	H_2N-collagen-NH_2 45°C[b]	POEGMEMA-*b*-collagen-*b*-POEGMEMA
POEGMEMA 64°C[a]	collagen-NH_2 45°C[b]	POEGMEMA-*b*-collagen

[a] LCST; [b] T_m of the triple-helix



Experimental

Materials. All chemicals were commercially available and used as received unless mentioned otherwise. 2,2'-Azobisisobutyronitrile (AIBN) was recrystallized from diethyl ether. Diethylene glycol methyl ether methacrylate (DEGMEMA) and oligoethylene glycol methyl ether methacrylate (OEGMEMA, M = 300 g/mol) were purified by distillation in vacuum. Tetrahydrofurane (THF) and dioxane for the polymerization, the radical substitution with AIBN, and the work-up of the polymers were distilled from sodium / potassium.

Instrumentation. ^{1}H-NMR spectra were recorded on a Bruker AC 300 MHz FT-NMR, a Bruker Avance II 400 FT-NMR or a Bruker Avance 600 FT-NMR spectrometer working at 300, 400 and 600 MHz, respectively, and ^{19}F-NMR spectra on a Bruker 376.5 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. For GPC in

N,N-dimethylformamide (DMF) containing 0.25 M LiBr an Agilent 1100 Series GPC set-up with three PSS HEMA columns ($10^6/10^5/10^4$ g/mol), a UV and a RI detector was used, which were calibrated using PMMA standards by PSS (Polymer Standards Service, Mainz). The eluent was used at 50°C and a flow rate of 1 mL/min. GPC in THF was performed on a GPC set-up consisting of the following components: a Jasco PU-1580 pump, a Jasco AS-1555 autosampler, MZ-Gel-SDplus columns (10^2, 10^4 and 10^6 Å^2), a Jasco UV-1575 UV/vis detector, and a Jasco RI-1530 refractive index detector. Polystyrene standards by PSS were used for calibration.

Synthesis of the collagen-like peptide with one amine end-group. The collagen-like peptide sequence with one amine end-group was obtained via the same solid-phase peptide synthesis (SPPS) procedure as described for the one with two amine end-groups.[1] Prior to cleavage from the resin, the original N-terminus was acetylated following a standard procedure, and thus, after cleavage using 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in dichloromethane (2 hours at room temperature), the collagen-like peptide exhibited only one free amine end-group. The peptide was isolated via precipitation from cold diethyl ether followed by lyophilization from a mixture of water and acetonitrile (50:50) and purification via reverse-phase high performance liquid chromatography (RP-HPLC) again followed by lyophilization from the same solvent mixture. Synthesis and verification of the desired structure were performed in the laboratory of [redacted] at the University of Delaware by [redacted]. Batch 1 (for PDEGMEMA-*b*-collagen) GPC (DMF): M_n = 3100 g/mol; M_w = 4000 g/mol; PDI = 1.26; batch 2 (for POEGMEMA- *b*-collagen): M_n = 3000 g/mol; M_w = 3700 g/mol; PDI = 1.25.

Synthesis of PDEGMEMA. PDEGMEMA was synthesized via RAFT polymerization using the PFP-CTA according to the same procedure as published previously[1] and working with the same ratios, so that the same molecular weights were obtained. Also, the dithioester end-group was substituted radically via conversion with an

excess of AIBN as described before.[1,12] ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.09 (br, 2nH); 3.67 (br, 2nH); 3.61 (br, 2nH); 3.55 (br, 2nH); 3.38 (br, 3nH); 2.87 (w, 2H); 2.10 – 1.70 (br, 2nH); 1.50 – 1.15 (w, 11H); 1.15 – 0.65 (br, 3nH); DP = 27, M = 5500 g/mol, calculated via end-group analysis in NMR; ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.57 (m, 2F); -157.49 (m, 1F); -161.98 (m, 2F); GPC (THF): M_n = 4500 g/mol; M_w = 5000 g/mol; PDI = 1.11; GPC (DMF): M_n = 2900 g/mol; M_w = 3500 g/mol; PDI = 1.19.

In some rare cases, a small higher molecular weight shoulder was observed in the GPC elugram of the polymer after conversion with AIBN. This higher molecular weight species eluted at a retention time corresponding to a molecular weight twice as high as the one of the desired species, so that dimer formation via recombination can be assumed to occur as a side reaction of the radical substitution reaction. In this case, the product was fractionated via precipitation in diethyl ether with an increasing amount of hexane added. That way, fractions with high dimer concentration were isolated at low hexane content, while the fractions precipitated with higher hexane content afterward were dimer-free.

Synthesis of the PDEGMEMA-*b*-collagen diblock copolymer. In a small reaction tube equipped with a stir bar, 14 mg (~ $2.57*10^{-6}$ mol) PDEGMEMA with an activated ester α-end-group were mixed with 6.2 mg ($1.28*10^{-6}$ mol) collagen-like peptide with one amine end-group in 1.3 mL dry DMF and roughly 1.5 µL triethylamine (at least 0.4 µL, equimolar amount with respect to the activated ester). The reaction tube was capped with a septum and equipped with a nitrogen balloon, before the clear, colorless solution was stirred in a preheated oil bath at 35°C for 3 days. The product was precipitated three times in cool diethyl ether. After drying in vacuum, 12.6 mg of the slightly yellowish, viscous product were obtained ($1.24*10^{-6}$ mol, 97%). GPC (DMF): M_n = 8800 g/mol; M_w = 11300 g/mol; PDI = 1.28.

Deprotection of the PDEGMEMA-*b*-collagen diblock copolymer. 11.6 mg of the PDEGMEMA-*b*-collagen diblock copolymer were dissolved in the deprotection

mixture, which was mixed in a separate vial before (945 µL TFA, 25 µL 1,2-ethanedithiol, 25 µL deionized water, and 10 µL triisopropylsilane), and stirred at room temperature in a small reaction tube equipped with a septum for 2.5 hours. Afterward, the reaction mixture was dried with a nitrogen stream almost completely and the product was redissolved in 0.25 mL freshly distilled THF and then precipitated in 3 mL of a cool mixture of diethyl ether and hexane (50:50). After a second analog precipitation, the white, solid product was dissolved in a mixture of Milli-Q water (~ 6 mL) and acetonitrile (~ 1 mL) in order to allow for lyophilization (quantitative yield). ^{1}H NMR (600 MHz, d_6-DMSO): δ (ppm) = 8.94; 8.75; 8.37; 8.28; 8.21; 8.14; 8.00; 7.92; 7.87; 7.81; 7.75; 7.66; 7.50; 7.30; 7.20; 7.18; 7.11; 7.01; 6.83; 6.38; 6.12; 4.78; 4.54; 4.48; 4.33; 4.21; 4.00; 3.87; 3.76; 3.69; 3.66; 3.59; 3.52; 3.44; 3.26; 3.14; 3.07; 3.05; 2.75; 2.41; 2.21; 2.07; 1.95; 1.86; 1.72; 1.50; 1.37; 1.33; 1.25; 1.15; 1.14; 1.10; 1.04; 0.94; 0.78.

Synthesis of POEGMEMA. In a dry Schlenk tube equipped with a stir bar, 1.60 g (5.33 mmol) of the monomer OEGMEMA, 118.5 mg (0.27 mmol) PFP-CTA, and 4.4 mg ($2.7*10^{-5}$ mol) AIBN were dissolved in 3.2 mL freshly distilled dioxane. After three freeze-pump-thaw cycles, the reaction tube was refilled with argon and the clear, pink reaction solution was stirred in a preheated oilbath at 70°C. The polymerization was stopped after 20 hours by immersion of the reaction tube into an ice bath. After adding 2.5 mL of freshly distilled THF to the solution, the product was isolated by threefold precipitation in cool hexane. The pink, viscous polymer was dried in vacuum, 1.55 g (90%) polymer were obtained. ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.84 (w, 2H); 7.48 (w, 1H); 7.32 (w, 2H); 4.05 (br, 2nH); 3.95 – 3.10 (br, 14nH); 3.34 (br, 3nH); 2.84 (w, 2H); 2.25 – 1.55 (br, 2nH); 1.50 – 1.15 (w, 5H); 1.15 – 0.60 (br, 3nH); DP = 27, M = 8550 g/mol, calculated via end-group analysis in NMR; ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.53 (m, 2F); -157.51 (m, 1F); -161.98 (m, 2F); GPC (THF): M_n = 4000 g/mol; M_w = 4600 g/mol; PDI = 1.14.

For radical substitution of the dithioester end-group, 505 mg POEGMEMA were dissolved in 12 mL freshly distilled dioxane in a dry round bottom flask. 207 mg (1.26 mmol) AIBN were added to the clear, pink solution and the flask was equipped with a stir bar, a septum and an argon balloon, before it was immerged into an oilbath preheated to 80°C. While the solution was stirred at 80°C for 3 hours, its pink color vanished completely. Afterwards, the solution was quickly cooled down to room temperature with an ice bath, and most of the solvent was evaporated using a rotary evaporator. The viscous product was redissolved in 2 mL freshly distilled THF and precipitated three times in a cool mixture of diethyl ether and hexane (50:50). The colorless product was dried in vacuum (411 mg; 81%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.05 (br, 2nH); 3.90 – 3.45 (br, 14nH); 3.35 (br, 3nH); 2.85 (w, 2H); 2.20 – 1.55 (br, 2nH); 1.55 – 1.15 (w, 11H); 1.15 – 0.65 (br, 3nH); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.53 (m, 2F); -157.52 (m, 1F); -161.99 (m, 2F); GPC (THF): M_n = 4000 g/mol; M_w = 4600 g/mol; PDI = 1.15; GPC (DMF): M_n = 4100 g/mol; M_w = 4700 g/mol; PDI = 1.14.

As discussed for PDEGMEMA, recombination can occur as a side reaction of the radical substitution of the dithioester end-group. If dimers of POEGMEMA were observed in GPC, they could also be separated from the desired product via fractionating precipitation in diethyl ether with increasing amounts of hexane added.

Synthesis of the POEGMEMA-*b*-collagen diblock copolymer. 30.4 mg ($3.56*10^{-6}$ mol) POEGMEMA, 7.35 mg ($1.52*10^{-6}$ mol) collagen-like peptide with one amine end-group, and rougly 2 µL triethylamine (at least 0.4 µL, equimolar amount with respect to the activated ester groups) were dissolved in 1.5 mL dry DMF. The reaction tube equipped with a stir bar was then capped with a septum and an argon balloon, and the colorless solution was stirred at 35°C for 3 days. For precipitation of the product, the reaction mixture was dropped into 10 mL of cool diethyl ether, which turned slightly turbid at the beginning, but was completely clear after addition of all the DMF solution (+ 0.4 mL DMF for rinsing the reaction tube). Upon addition

of 10 mL of cool diethyl ether, the product precipitated, but after centrifugation at 10°C, only a very small amount of viscous precipitate did settle at the bottom of the centrifuge glass. Therefore, the ether was evaporated with a nitrogen stream, and from the remaining DMF solution, the product could be precipitated in a cold mixture of 10 mL diethyl ether and 1 mL hexane. After centrifugation at 10°C, the supernatant (fraction B) was transferred to another centrifuge glass for further investigation. The isolated polymer (fraction A) was redissolved in 0.25 mL dry DMF and precipitated two more times in a cold mixture of the same composition (10:1). After drying in vacuum, 19.4 mg (86%) colorless product were obtained. To test, if the supernatant of the first successful precipitation step (fraction B) contained more of the product, 6 mL cool hexane were added and the precipitate was isolated by centrifugation. It was redissolved in 0.2 mL dry DMF and precipitated twice in a cool mixture of 8 mL diethyl ether and 4 mL hexane. The dried polymer (13.1 mg) was analyzed by GPC. Besides the excess homopolymer, this fraction also contained some diblock copolymer. Fraction A: GPC (DMF): M_n = 9400 g/mol; M_w = 12900 g/mol; PDI = 1.37.

Deprotection of the POEGMEMA-*b*-collagen diblock copolymer. First, 1890 μL TFA, 50 μL 1,2-ethanedithiol, 50 μL deionized water, and 20 μL triisopropylsilane were mixed and then used to dissolve 17.7 mg of the POEGMEMA-*b*-collagen diblock copolymer. This solution was stirred in a small glass vial for 2.5 hours, before most of the mixture was evaporated using a nitrogen stream. The remaining slightly yellowish product was dissolved in 0.25 mL freshly distilled THF and dropped into 3 mL of a cold mixture of diethyl ether and hexane (50:50) for precipitation and separated from the solvents by centrifugation below 15°C. After three precipitation steps, the solid product was dried with a nitrogen stream and then dissolved in 5 mL Milli-Q water and 1 mL acetonitrile for lyophilization. 12.7 mg of the off-white, solid product were obtained after lyophilization (yield: 77%). ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) = 8.79; 8.39; 8.31; 8.22; 8.13; 8.12; 8.07; 8.03; 7.98; 7.90; 7.88; 7.86;

7.81; 7.66; 7.55; 7.53; 7.28; 7.23; 7.10; 6.97; 6.66; 4.79; 4.54; 4.48; 4.33; 4.26; 4.20; 4.00; 3.85; 3.80; 3,73; 3.68; 3.59; 3.52; 3.42; 3.24; 3.18; 3.06; 3.03; 2.76; 2.65; 2.32; 2.22; 2.07; 2.06; 1.87; 1.84; 1.73; 1.50; 1.33; 1.22; 1.14; 1.10; 1.04; 0.94; 0.77.

Synthesis of the collagen-like peptide with two amine end-groups. The synthesis of this collagen-like peptide with two amine end-groups was performed following the previously described procedure.[1] ESI-MS: MW = 4786.9 g/mol (m/z = 1595.5 [$(M+3H)^{3+}$, calc: 1595.8]). GPC (DMF): M_n = 2300 g/mol, M_w = 3100 g/mol, PDI = 1.35.

Synthesis of the POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer. In a dry reaction tube, 36.8 mg ($4.31*10^{-6}$ mol) POEGMEMA, 4.4 mg ($9.19*10^{-7}$ mol) collagen-like peptide with two amine end-groups, roughly 2 µL triethylamine (at least 0.5 µL, equimolar amount with respect to the activated ester groups) and 1.5 mL dry DMF were mixed. The reaction tube was equipped with a stir bar, a septum and an argon balloon, and the colorless solution was stirred at 35°C for 3 days. Afterwards, the reaction mixture was dropped into 10 mL of cool diethyl ether in order to precipitate the product. The ether turned slightly turbid with the first drops, but when all the solution was added, the mixture was completely clear again. Upon adding more diethyl ether (10 mL), the mixture turned turbid, but after centrifugation at 10°C, all the precipitate was dissolved again. Therefore, the ether was evaporated completely with a nitrogen stream and the remaining solution was dropped into a cold mixture of 10 mL diethyl ether and 1 mL hexane, in which the product could successfully be precipitated. After centrifugation at 10°C, viscous polymer (fraction A) was obtained at the bottom of the centrifuge glass. The supernatant was separated from the product and the solvent was evaporated in order to allow for further characterization of the polymer fraction (B) dissolved in the supernatant. After another precipitation step in 10 mL hexane and 6 mL diethyl ether, the GPC elugram of this fraction B indicated, that the supernatant contained almost only homopolymer (POEGMEMA). The product fraction (A) isolated in the

first precipitation was redissolved in 0.2 mL dry DMF and precipitated from a cold mixture of 2 mL diethyl ether and 1 mL hexane. The obtained colorless product was dried in vacuum (yield: 13.2 mg, 58%). GPC (DMF): M_n = 8900 g/mol; M_w = 15500 g/mol; PDI = 1.74.

Deprotection of the POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer. 11.5 mg of the POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer were deprotected following the same procedure as described before for the POEGMEMA-*b*-collagen diblock copolymer. After lyophilization, 9.3 mg (85%) slightly yellowish, solid product were obtained. Taking the high molecular weight of this hybrid into consideration, the obtained mass was too little to measure a well dissolved NMR spectrum.

Conclusion

Summarizing the syntheses of the four realized polymer-peptide-conjugates, more precisely the polymer-*b*-collagen diblock copolymers and the polymer-*b*-collagen-*b*-polymer triblock copolymers with PDEGMEMA as well as POEGMEMA as polymer blocks, the general applicability of this bioconjugation approach for polymers with a reactive pentafluorophenyl ester α-end-group was demonstrated. In all the four examples, the conversions of this activated ester end-group with the amine end-groups of the respective peptide were highly efficient and the excess polymer building block could be separated from the product successfully. Moreover, the described biohybrids are expected to show double thermo-responsive behavior in aqueous solution, as already observed on the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer. The polymer blocks exhibit an individual LCST and should collapse upon heating, while the collagen-like peptide is known to form triple helices, which can be unfolded thermally. The synthesized block copolymers are promising candidates to study the mutual effect of these two phenomena on each

other and to learn, which supramolecular structures result from the double thermo-responsive character.

Acknowledgement

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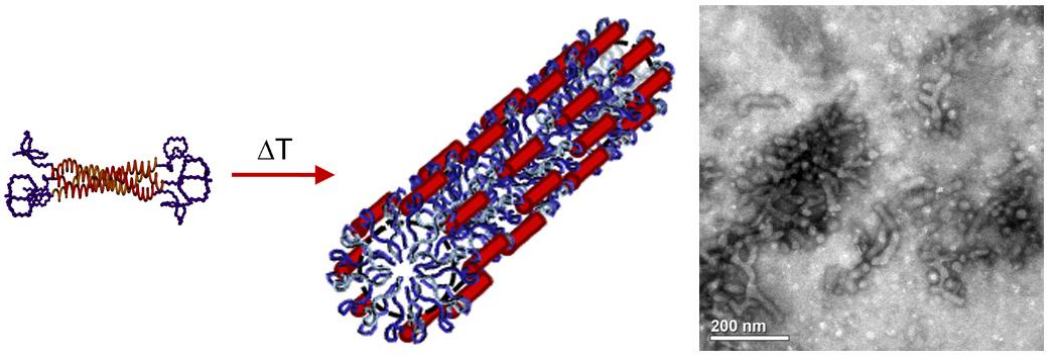
4.3.3 Self-assembly of Double Thermo-responsive Polymer-Peptide-Conjugates on the Basis of a Collagen-like Peptide and Poly(diethylene glycol methyl ether methacrylate)



Introduction

Bioconjugates consisting of synthetic polymer blocks and peptide blocks very often show an interesting self-assembly behavior in solution,[1] which differs significantly from the behavior of purely synthetic block copolymers in solution. Besides differences in polarity in comparison to the polymer block, many peptide segments show a high potential to drive the self-assembly behavior of such bioconjugates toward the formation of hierarchically organized nanoscale structures, because they have a high tendency to fold into secondary or even higher order structures. In addition, superstructures on the basis of such bioconjugates often undergo size or shape transitions upon the application of an external stimulus because of the stimuli-responsive properties of either the biological or the synthetic entity.

In this context, investigation of bioconjugates composed of the thermo-responsive poly(diethylene glycol methyl ether methacrylate), briefly PDEGMEMA,[2] and the collagen-like peptide sequence[3-5] with the tendency to assemble into triple-helices promises to reveal unusual self-assemblies. Therefore, a PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer and a PDEGMEMA-*b*-collagen diblock copolymer were synthesized via conjugation of the independently prepared building blocks

applying activated ester chemistry.[6] These two different bioconjugates are not only expected to show different lower critical solution temperatures (LCST) in water, but also to result in possibly completely different temperature-responsive superstructures.

Results and Discussion

As already discussed in chapter 4.3.1, circular dichroism (CD) spectroscopy confirmed that the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer self-assembled into supramolecular structures containing collagen-like triple-helices at low temperatures in aqueous solution.[6] Moreover, it was indicated that the unfolding of these polymer conjugated triple-helices upon heating proceeded as a multi-step transition (see **Figure 3b** in chapter 4.3.1) opposite to what is known for the pure collagen-like peptide showing a cooperative unfolding indicated by a sigmoidal denaturation curve.[3-5] Since the synthetic polymer blocks consist of the thermo-responsive PDEGMEMA, it is likely that this gradual transition of the copolymer involves steps mainly relying on the temperature-dependent collapse of the polymer blocks as well as steps caused mainly by the "melting"of the collagen-like triple-helix. However, CD spectroscopy probes only changes in the secondary structure of the peptide block. To learn more about the overall changes in the supramolecular structure, turbidity measurements were performed studying the macroscopically observable transitions.

The change of the turbidity of an aqueous solution of the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer (c = 3.2 mg/mL) was monitored as a function of temperature heating from 10°C (after an equilibration period of 30 minutes at 10°C under constant stirring) to 80°C with a heating rate of 1°C/min. Afterward, the solution was cooled down from 80°C back to 10°C with a cooling rate of -1°C/min. In **Figure 7**, the relative transmittance is plotted versus temperature for three heating and cooling cycles (in between the end of one cooling period and the beginning of

the next heating period, the sample was given time for equilibration of 45 minutes at 10°C under stirring).

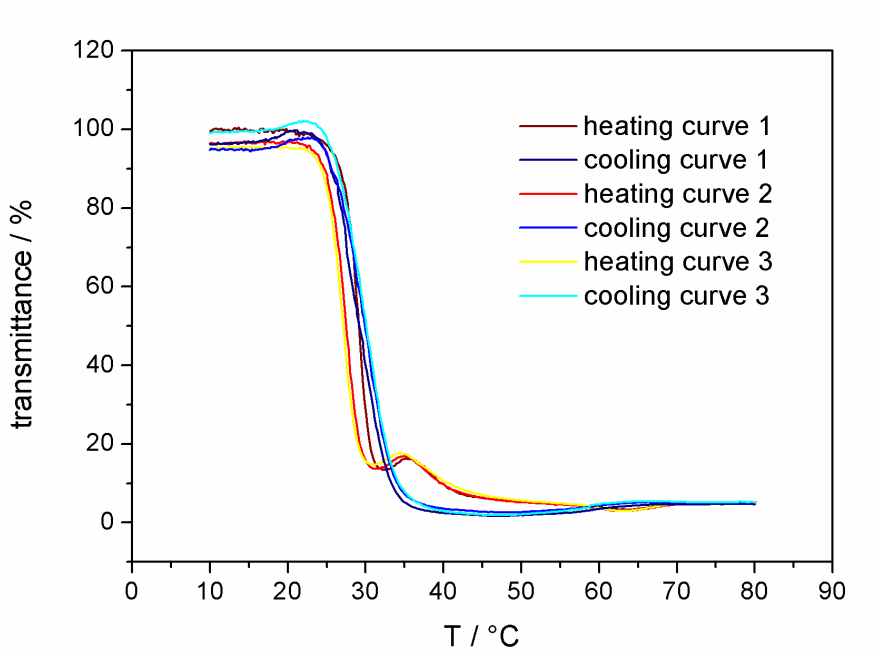


FIGURE 7: Relative transmittance of a solution of the PDEGMEMA-b-collagen-b-PDEGMEMA triblock copolymer as a function of temperature.

The transmittance of the almost completely clear solution was set to 100% at the beginning of the first temperature cycle. From this initial value, transmittance started to decrease at 24°C and then dropped rapidly to 14% upon heating the sample to 31-32°C. At 34-35°C, the heating curve exhibited a small local maximum, at which the transmittance reached 17% again, before it further decreased to less than 10% between 36-40°C and to less than 5% above 50°C. These observations indicate that the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer underwent at least two transitions upon heating, as already suggested by the CD denaturation curve (see **Figure 4b** in chapter 4.3.1). The onset of the first transition, presumably the formation of larger aggregates due to the collapse of the PDEGMEMA blocks at its LCST, was observed around 24°C, which is a lower temperature than the cloud point determined in the very first measurement (see chapter 4.3.1). This can be explained by the concentration dependency of the cloud point at such low concentrations as used in the first experiment (the cloud point is usually reached at higher

temperatures for low concentrations). With the choice of a concentration higher than 3 mg/mL, the results discussed here were collected in a concentration range, in which the cloud point of PDEGMEMA does not change significantly upon an increase in concentration.[2] Also, instead of a 0.2 mm sample cell, a 10 mm sample cell was used in the experiment described here, which allows for more accurate temperature control, because all the four walls of the larger sample cell are in direct contact with the heating/cooling element.

The cooling curve was measured directly following the heating curve, and the transmittance did not change significantly, until a temperature of 34°C was reached. Here, transmittance increased from 5% to 10% within a temperature difference of only 1°C, and then, the turbidity curve underwent a steep increase up to 99% transmittance at 23°C. Here, the curve exhibited a maximum (23-20°C) and below 20°C, the transmittance dropped slightly, before it stayed constant at about 96% for the rest of the cooling curve. Thus, the solution recovered full transmittance (tolerating a small deviation of less than 5% from the initial value). A second and a third temperature cycle confirmed reproducibility and reversibility of the described transitions.

For more detailed studies of the self-assembly behavior of the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer on a smaller size scale, dynamic light scattering experiments were carried out. These measurements could not be conducted under continuous temperature variation and so distinct temperatures were chosen on the basis of the heating and cooling curves in **Figure 7**, at which defined aggregation states were expected, i.e. at 10°C for examination of the initial state, at 31°C representing the state after the first sharp phase transition, at 34°C to study the possibly following second transition, at 50°C as a state after all these transitions (and also as a temperature higher than the melting temperature of the collagen-like helices of pure peptide), and finally at 23°C because of the maximum in the cooling curves.

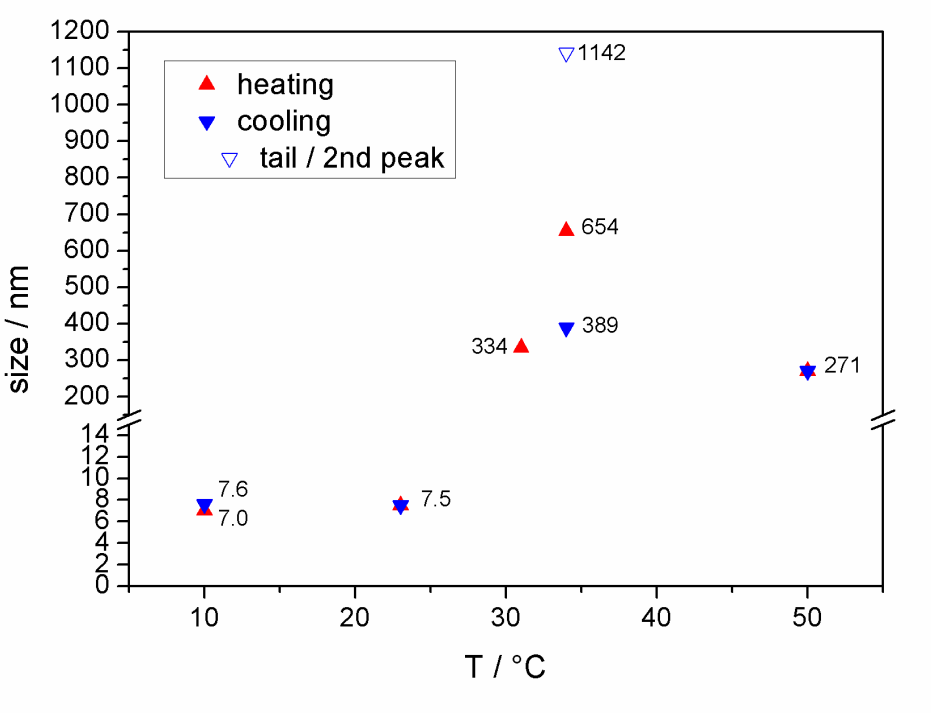


FIGURE 8: Sizes of the supramolecular structures formed by the PDEGMEMA-b-collagen-b-PDEGMEMA triblock copolymer obtained via DLS.

The sizes of the supramolecular structures formed by the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer in aqueous solution (c = 1 mg/mL, lower concentrations did not give reproducible results, at higher concentrations the solutions were not 100% optically clear and thus inappropriate for DLS measurements) are displayed in **Figure 8** as a function of temperature. In the initial state at 10°C, structures with a diameter of about 7.0 nm were found and the size distribution was relatively narrow (full width at half maximum, FWHM = 3 nm). At 23°C, almost the same size distribution was observed with an average diameter of 7.5 nm (FWHM = 3 nm). Upon heating to higher temperatures, much larger, and less uniform aggregates were formed with more than 300 nm in diameter (334 nm, FWHM = 200 nm) at 31°C and even more than 600 nm (654 nm, FWHM = 360 nm) at 34°C. However, the structures found at 50°C had an average diameter of only 271 nm (FWHM = 150 nm). After these measurements, the same solution was also analyzed during cooling back down to 10°C. At a temperature of 34°C, a bimodal size distribution was observed. The larger fraction of the aggregates was measured to have a size of about 389 nm (FWHM = 165 nm), but in each measurement either a

second species of more than 1000 nm in size or at least a higher molecular weight tail was found in addition to the described smaller size distribution. This observation was ascribed to a non-systematic breakup of the aggregates and was the reason, why the size distribution at 31°C was not further analyzed. The measurements at 23°C and 10°C, both showed narrow size distributions around 7.5 nm (FWHM = 3 nm), thus almost exactly reflected the initial size values, indicating reversibility of the overall transition. However, there was no evidence for the maximum observed at 23°C in turbidimetry. It should also be mentioned, that the software used for analysis of the scattering data automatically assumes a spherical shape of particles, which does not necessarily hold true for all kinds of possible aggregates. Hence, the values obtained from this measurement are rather an indication for the size range of the supramolecular structures formed than an exact diameter.

Parallel to these experiments, the self-assembly of the triblock copolymer was also investigated via transmission electron microscopy (TEM) by our cooperation partners. Solutions of the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer in phosphate buffered saline (PBS) were heated to 20°C and 37°C, respectively, before they were dropped rapidly onto a TEM grid.

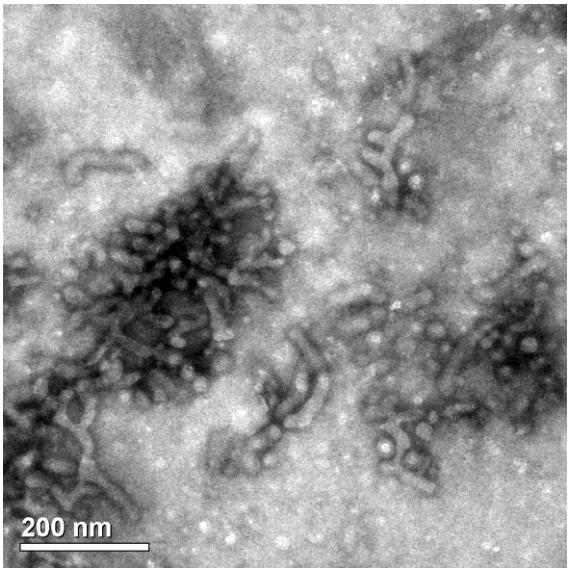


FIGURE 9: TEM image of the supramolecular structures formed by the PDEGMEMA-b-collagen-b-PDEGMEMA triblock copolymer at 37°C in PBS.

In the case of the solution kept at 20°C, and thus below the LCST of the polymer blocks, no superstructures could be found, while the second experiment with preparation at 37°C revealed worm-like and spherical superstructures (**Figure 9**).

On the basis of the described measurements, the following hypotheses on the temperature-dependent self-assembly behavior of the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer in aqueous solution are proposed and depicted in **Figure 10**. In the initial state, i.e. below the LCST of PDEGMEMA, the triblock copolymer exists as a water-soluble trimer, in which the peptide blocks form a collagen-like triple-helix (peptides oriented parallel to each other). When the temperature is increased to more than 24°C, the PDEGMEMA blocks start to collapse and rigid amphiphilic structures are formed. The hydrophobic interactions between the PDEGMEMA blocks are supposed to cause aggregation of more than three triblock copolymers into larger supramolecular assemblies. This is also responsible for the decreasing transmittance detected at temperatures above 24°C. According to the results from CD spectroscopy, the secondary structure of the peptide blocks is not yet changing significantly at these temperatures, so that the peptide block can be expected to be a stiff, rod-like moiety with a hydrophilic character. The large aggregates suggested by the DLS results at 31°C and 34°C as well as by the TEM image of the sample prepared from a solution at 37°C, are supposed to have a worm-like shape preferentially. One could imagine, that the hydrophobic polymer blocks prefer the intra- and intermolecular interactions among PDEGMEMA blocks and therefore assemble in the core of either spherical or worm-like micelles, while the hydrophilic peptide blocks are exposed on the outside of the superstructures. Due to the stiff, rod-like character of the triple-helices, they are likely to promote the formation objects with a form anisotropy, such as worm-like micelles with the longitudinal axes of the triple-helices oriented perpendicular or parallel to the longitudinal axis of the worm-like micelles (**see Figure 10**) or the self-organization into large spherical micelles, so that the rod-like peptide trimers do not need to be bent.

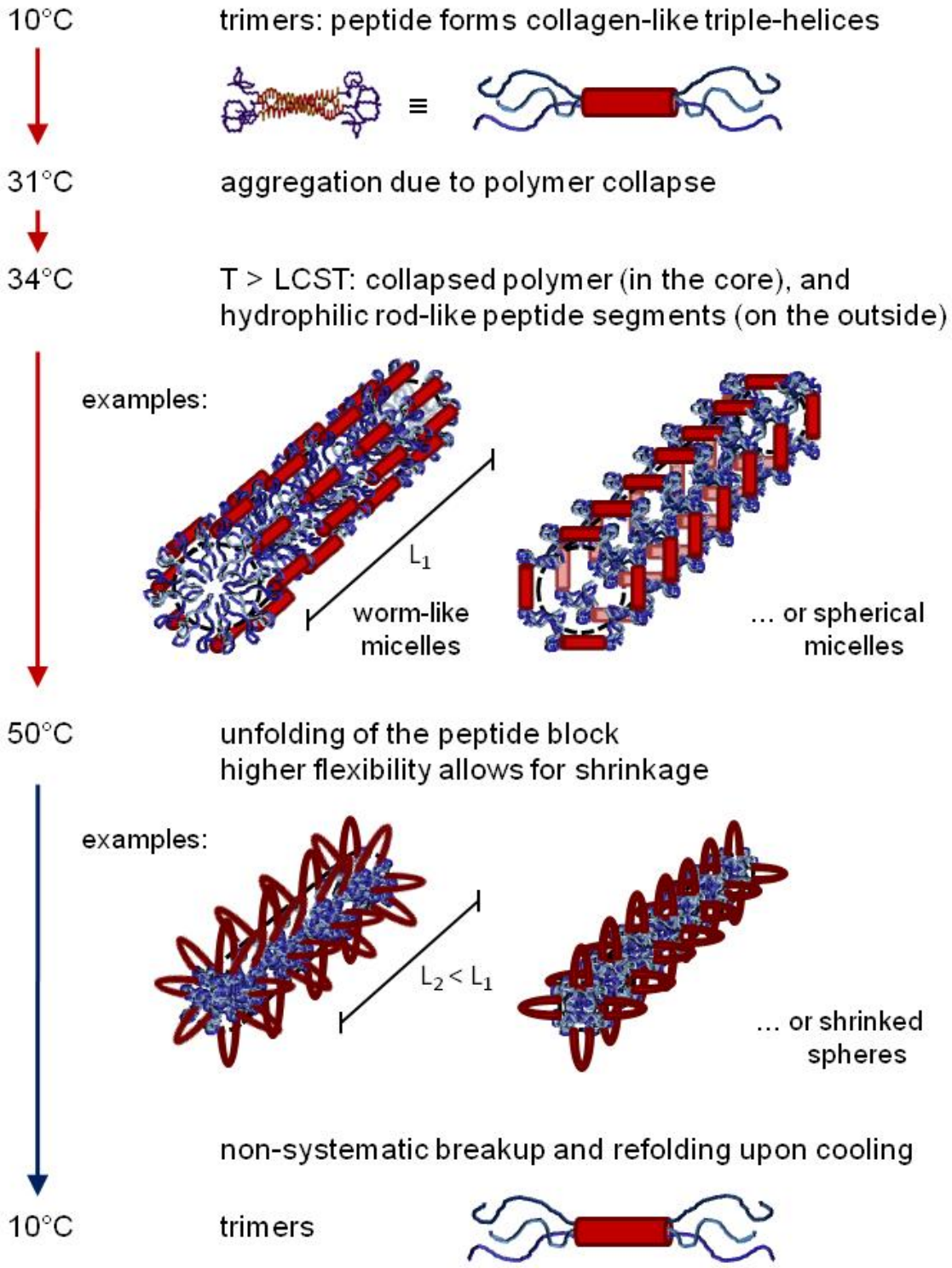


*FIGURE 10: Illustration of the hypotheses on the temperature-dependent self-assembly behavior of the PDEGMEMA-**b**-collagen-**b**-PDEGMEMA copolymer.*

The broad size distributions determined in the DLS experiments at 31°C and 34°C could easily be explained by both, the coexistence of worm-like and spherical micelles as well as different lengths of the worm-like structures. Moreover, in the DLS experiment at 50°C, a decrease in particle size was observed with respect to the results at 34°C, so that a second transition can be postulated. This is likely to be caused by the temperature induced unfolding of the collagen-like triple-helices confirmed by CD spectroscopy. Assuming the denaturation of the helical secondary structure, the peptide blocks would become more flexible and thus allow for shrinkage of the worm-like structures, as illustrated in **Figure 10**. The polydispersity in size determined via DLS at 34°C during the subsequent cooling of the sample indicates a non-systematic breakup of the aggregates. However, it can be assumed, that the peptide blocks refold into collagen-like triple-helices upon cooling and that the PDEGMEMA is redissolved at temperatures below 24°C, so that the initial trimers were recovered after a full temperature cycle. From the described results, it cannot be decided unambiguously, at which exact temperature (23°C or lower) the trimers with well-defined collagen-like triple-helices are assembled and refolded completely. For more detailed understanding of these kinetic details, temperature and also time-dependent CD experiments could be helpful. Also, our cooperation partners are working on further TEM and Cryo-TEM experiments, to learn more about the shape of the two types of aggregates observed via DLS at 34°C and 50°C, but these challenging studies are beyond the scope of this thesis.

As a second bioconjugate, the PDEGMEMA-*b*-collagen diblock copolymer and its thermo-responisve self-assembly behavior were investigated. As expected, CD spectroscopy on a cool solution of the PDEGMEMA-*b*-collagen diblock copolymer in PBS confirmed that the peptide blocks of this copolymer also assembled into structures containing collagen-like triple-helices.[7] Since the three peptide strands in such a triple-helix are usually oriented parallel to each other, all the three PDEGMEMA blocks should presumably be found at one end of such a triple-helix.

Figure 11 shows the results from turbidity measurements performed on a solution of the PDEGMEMA-*b*-collagen diblock copolymer in Milli-Q water (c = 5.2 mg/mL) after an equilibration period of 30 minutes at 10°C under continuous stirring, after which the initial value of transmittance was set to 100%. Heating and cooling curves of one temperature cycle were recorded in a direct sequence with a rate of ±1°C/min, and between one cycle and the next one, the solution was given 45 minutes at 10°C under stirring for equilibration.

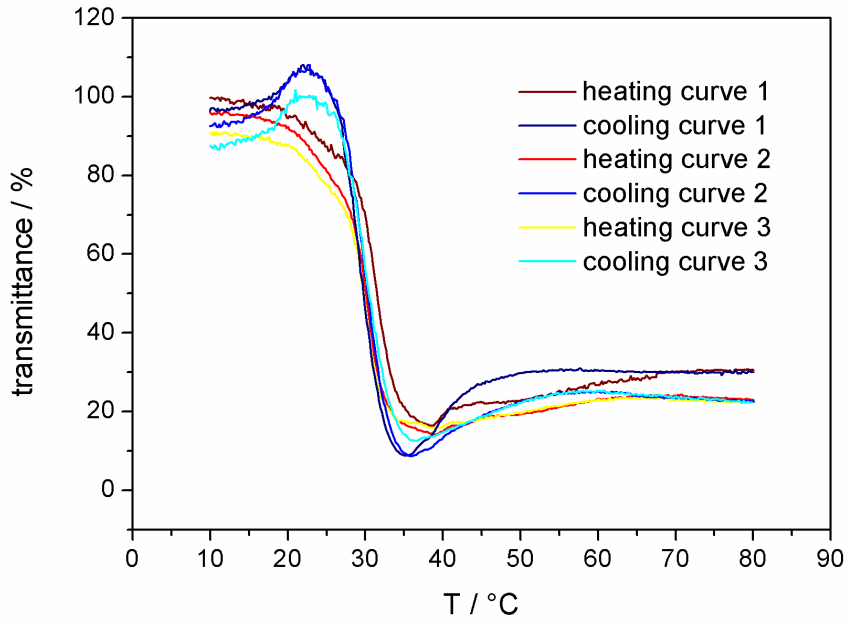


FIGURE 11: Relative transmittance of a solution of the PDEGMEMA-b-collagen diblock copolymer as a function of temperature.

As expected, the hydrophilic peptide block had a stronger influence on the overall polarity of the diblock copolymer than in the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer, so that the macroscopic phase separation was shifted toward slightly higher temperatures in comparison to the triblock copolymer. However, especially the heating curve of the diblock copolymer looked different from the one of the triblock copolymer. The transmittance of the PDEGMEMA-*b*-collagen diblock copolymer solution decreased already a little bit at temperatures above 21°C, but then dropped significantly above 27°C, to reach its minimum between 36°C and 40°C (roughly 15%). At higher temperatures, transmittance increased again a little bit to values between 22% and 30%. During the subsequent

cooling periods, transmittance did not change significantly first, but below 50°C it decreased to its minimum of about 10% at 36°C, to then undergo a steep increase up to 23-22°C. Here, the cooling curves exhibited their maximum and then went down to values somewhat lower than the initial values of the respective heating curves.

The general course of the heating and cooling curves was reproducible over all three conducted temperature cycles and thus gave reason to look closer at the sizes of the formed superstructures at the following distinct temperatures: Dynamic light scattering experiments were performed at 10°C to study the initial state, at 24.5°C representing the first slight transition state, at 36°C, the minimum of transmittance, at 55°C and thus above the last observable transition during heating, and at 23°C because of the maximum of the cooling curves. The average sizes of the superstructures formed by the PDEGMEMA-*b*-collagen diblock copolymer in aqueous solution (c = 0.99 mg/mL, solution optically clear at 10°C), as they were determined via DLS, are plotted in **Figure 12**. At 10°C, small supramolecular structures were found with an average diameter of 5.7 nm and a narrow size distribution (FWHM = 3 nm), and thus, as expected, a little bit smaller than the initial state found for the triblock copolymer. Almost the same result was obtained at 23°C (6.8 nm, FWHM = 3 nm) and at 24.5°C (4.7 nm, FWHM = 2 nm), but at 24.5°C, some of the measurements revealed bimodal size distributions showing a small additional peak at 8.7 nm. In contrast to these small structures, an average size of 463 nm with a broad size distribution (FWHM = 330 nm) was determined for the diblock copolymer solution at 36°C. Thus, large aggregates must have formed, which then decomposed into smaller superstructures upon further heating (50°C: 175 nm, FWHM = 90 nm). These results corresponded well to the strong decrease in transmittance up to 36°C and the subsequent increase at higher temperatures, observed in turbidimetry. When cooling down the diblock copolymer solution to 36°C, structures with a size of roughly 723 nm and very broad size distribution were found (FWHM = 460 nm), which was again in good agreement with turbidimetry. When the temperature was lowered to 24.5°C, the observed average diameter came back to a value of 5.7 nm

(FWHM = 2 nm) and thus the size of the initial state before heating. At 23°C and at 10°C, very similar results were obtained (23°C: 5.4 nm, FWHM = 2 nm; 10°C: 4.4 nm, FWHM = 2 nm).

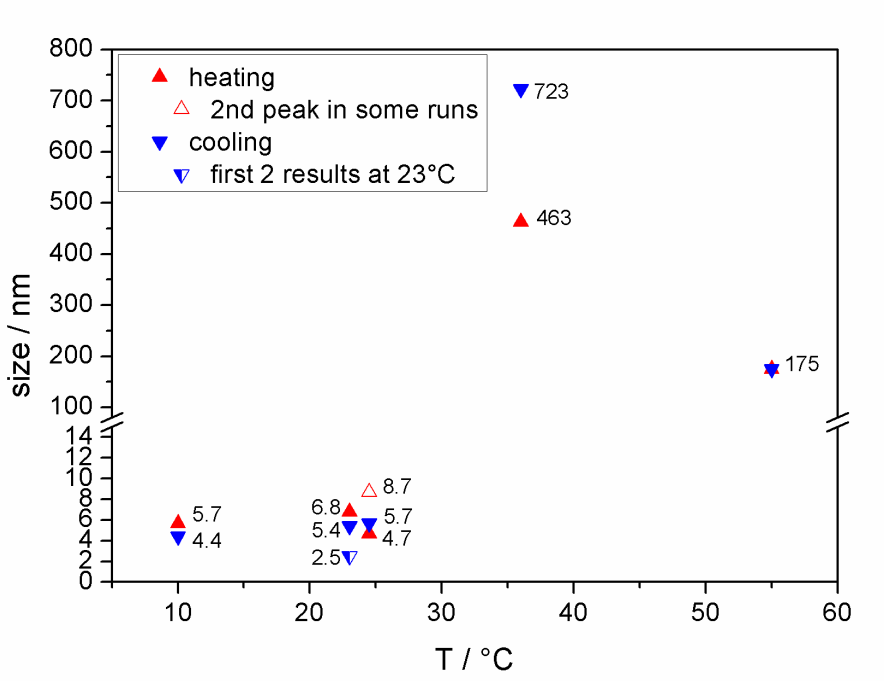


FIGURE 12: Sizes of the supramolecular structures formed by the PDEGMEMA-b-collagen diblock copolymer obtained via DLS.

In one of the first measurements, when the equilibration period of 10 minutes at a new temperature was not yet established, an interesting observation was made: The first two values measured after 2 minutes at 23°C (cooling) were much lower (2.5 nm in average) and more uniform (FWHM = 1 nm) than the following values, which increased with time to a constant value of 5.4 nm in average. This should be kept in mind, when comparing the DLS results to the turbidity measurements, which were recorded under continuous heating and cooling with a rate as high as 1°C/min.

Summarizing the results obtained via turbidimetry and DLS, the self-assembly behavior of the PDEGMEMA-*b*-collagen diblock copolymer in aqueous solution can be envisioned possibly as described in the following hypotheses. Starting from a triple-helical trimer at low temperatures, it can be assumed, that the first slight changes in transmittance as well as in size (at 24.5°C) arise from the beginning of the polymer collapse. Depending on the concentration of the probed solution, this could

lead to smaller trimers with contracted PDEGMEMA blocks or initial aggregation of only a few trimers via their hydrophobic polymer blocks in the early stage of this transition. While the decrease in transmittance at a concentration of 5.2 mg/mL supports the theory of small aggregates of a few trimers, the DLS results allow for the interpretation, that both types of superstructures, but mostly the contracted trimers could occur at this low concentration. At higher temperatures, both measurements are in good agreement and it is very likely, that large polydisperse aggregates are formed above the LCST of PDEGMEMA, so that the hydrophobic polymer blocks can maximize their intermolecular interaction and the hydrophilic peptide helices are oriented toward the outside. The high value obtained in the DLS experiment at 36°C suggests that these aggregates are even larger than normal micelles. This could possibly be explained by weak intermicellar interactions among peptide blocks from different micelles, which lead to even larger superstructures, as illustrated in **Figure 13**. Assuming that these peptide-peptide interactions are related to the triple-helix formation, the observation of increasing transmittance and decreasing particle size above 50°C would speak for the unfolding of the peptide helices at these temperatures, which leads to disassembly of the multi-micellar structures and results in separate classical micelles of the amphiphilic diblock copolymer with unfolded hydrophilic peptide blocks on the outside and hydrophobic PDEGMEMA in the core of the micelles. This transition seems to be more or less reversible, when taking the significant drop of transmittance in the cooling curve and the strong increase in particle size upon cooling into consideration. However, DLS showed even larger aggregates at 36°C, when cooling down the solution, than at the same temperature during heating. This could be explained by an imperfect refolding of the collagen-like helices at these relatively high temperatures (in comparison to the low temperature of 10°C, which is usually needed for complete folding of the triple-helices) leading to less ordered and thus larger superstructures. And finally, the results at temperatures below the LCST of the polymer block indicate recovery of the trimers containing collagen-like triple-helices. Further, it is

imaginable, that before the initial trimeric state was reformed, single diblock copolymers existed for a short time, which would explain the first low values measured at 23°C, but more detailed kinetic studies, for example via CD spectroscopy, are probably necessary to support this theory.

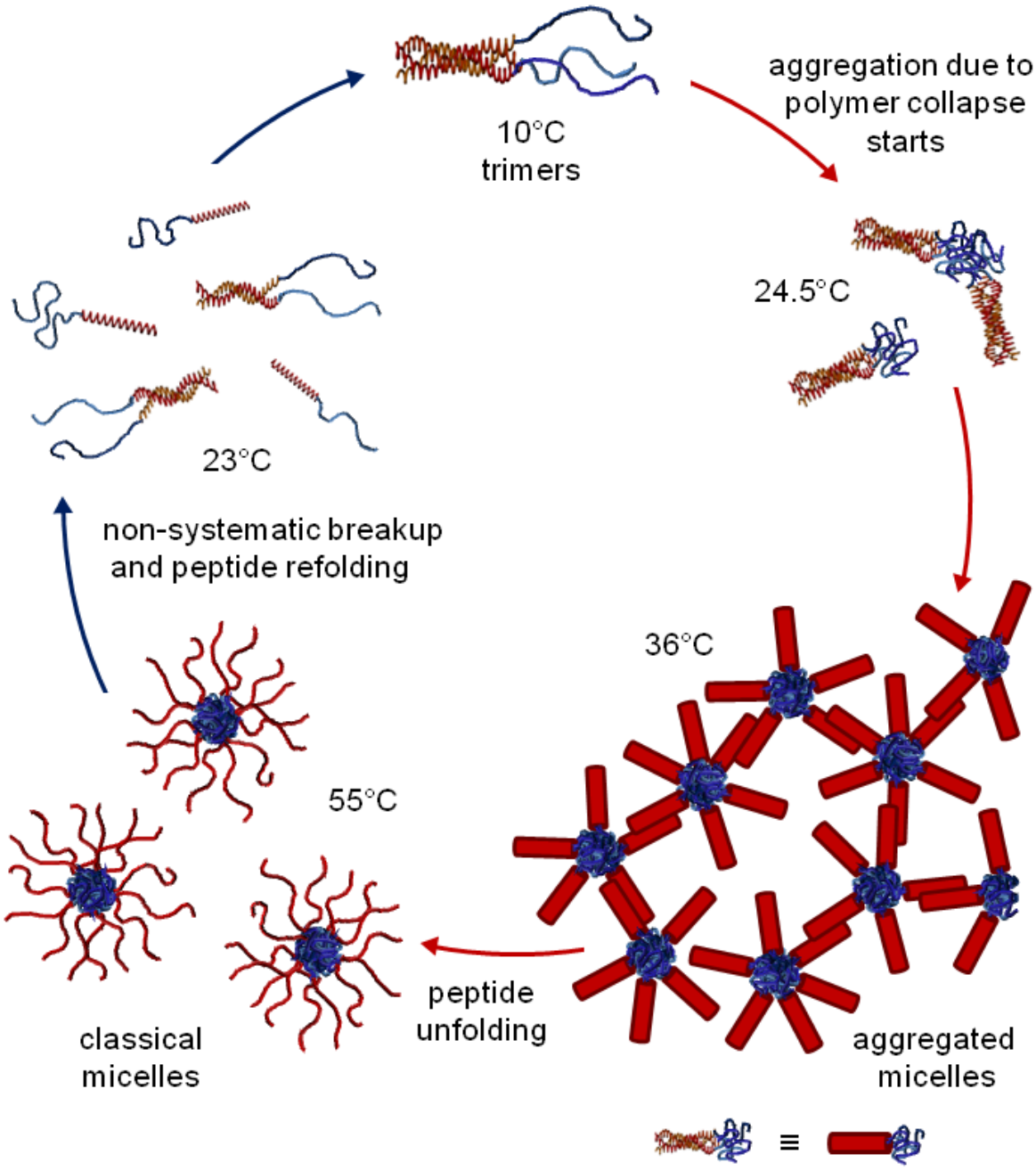


*FIGURE 13: Illustration of the hypotheses on the temperature-dependent self-assembly behavior of the PDEGMEMA-**b**-collagen copolymer.*

The PDEGMEMA-based di- and triblock copolymers are nice examples for double stimuli-responsive bioconjugates: Upon a first external stimulation, i.e. the increase in temperature in this case addressing the PDEGMEMA block(s), they self-assemble into supramolecular structures, which can afterward be altered by a second stimulation addressing the peptide block. Analog hybrid systems with synthetic polymer blocks exhibiting an LCST higher than the melting temperature of the collagen-like peptide should be ideal candidates to study the self-assembly behavior for the case, in which the two described stimuli-responsive building blocks are addressed in the inverse order. Therefor, di- and triblock copolymers consisting of the collagen-like peptide and POEGMEMA with an LCST around 64°C were synthesized.

The preparation of a solution of the POEGMEMA-*b*-collagen-*b*-POEGMEMA triblock copolymer (c = 3.1 mg/mL) for turbidity measurements already revealed a better water-solubility of this bioconjugate in comparison to the PDEGMEMA-based copolymers and the actual measurements showed, that it is that well soluble in water, that the phase-transition could not be monitored completely within the chosen temperature window (10°C – 85°C). Thus, the experiment was repeated within a temperature window of 10°C to 95°C (after an equilibration period of 45 minutes at 10°C, see **Figure 14**), but the phase-transition was still not complete at 95°C. That is, why no further turbidimetric studies were conducted on the diblock copolymer, which was expected to exhibit an even higher overall hydrophilicity than the triblock copolymer.

However, turbidimetry confirmed the assumption that the LCSTs of these hybrids systems should be higher temperatures than the LCSTs of the PDEGMEMA-based copolymers. These results motivate for further investigations of their ability to form trimers based on collagen-like triple-helices and stimuli-responsive supramolecular structures via CD spectroscopy and dynamic light scattering.

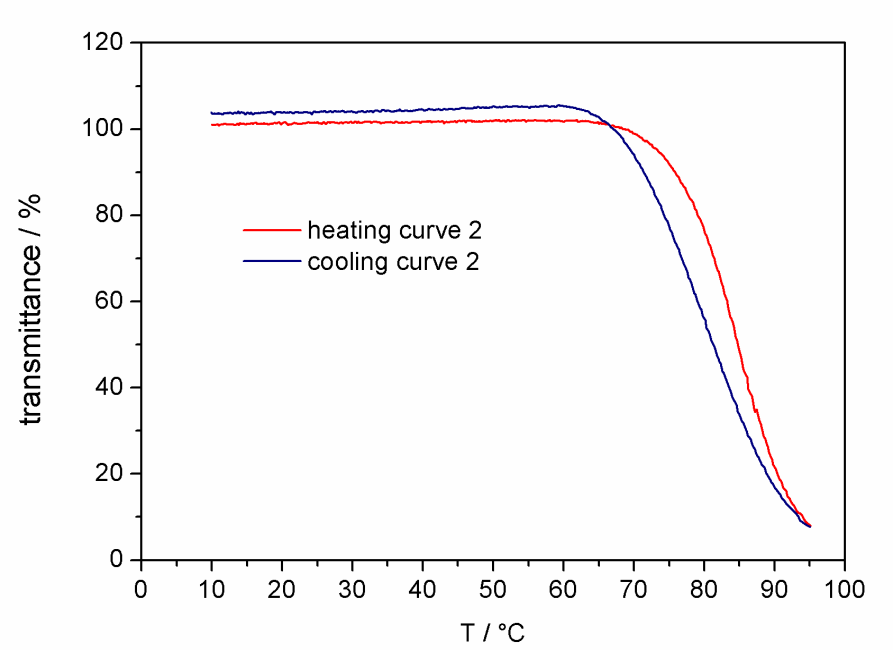


*FIGURE 14: Relative transmittance of a solution of the POEGMEMA-**b**-collagen-**b**-POEGMEMA triblock copolymer as a function of temperature.*

Experimental

Turbidimetry. Optical transmittance of a laser light beam (λ = 632 nm) through a 10 mm sample cell with the solution to be probed was measured using a Jasco V-630 spectrophotometer equipped with a Jasco ETC-717 Peletier element in order to determine turbidity as a function of temperature. The tested solution was heated and cooled at a rate of ±1°C/min under constant stirring with a small magnetic stir bar, while transmittance was detected in intervals of 0.2°C. All the sample solutions were prepared from lyophilized samples dissolved in Milli-Q water.

Dynamic Light Scattering. Sizes of the supramolecular structures were determined via dynamic light scattering (DLS) performed on a MALVERN Zetasizer Nano-S Size using a 40 μL quartz cuvette, He-Ne-laser light with a wavelength of λ = 633 nm, and a detection angle of 90°. An equilibration time of 10 minutes was given at each new temperature, before the first measurement was started, and each measurement consisted of 10 scans (10 seconds each). Values given in **Figures 8 and 12** represent the average of the peak maxima of at least 5 measurements with 60 seconds between

the end of one measurement and the beginning of the next one. The measured scattering intensities were converted into particle sizes (size distributions by number versus size) via the MALVERN software "Dispersion Technology System". All the sample solutions were prepared from lyophilized samples dissolved in Milli-Q water and were filtered through a 5 μm PTFE filter first and then through a hydrophilized 0.2 μm filter prior to the DLS experiments.

Conclusion

Both, the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer as well as the PDEGMEMA-*b*-collagen diblock copolymer form supramolecular structures containing collagen-like triple-helices in cool aqueous solutions, as confirmed via CD spectroscopy. Turbidimetry and dynamic light scattering indicated that they self-assemble into double stimuli-responsive superstructures in the size range of several hundred nanometers upon heating to temperatures close to body temperature. In the case of the triblock copolymer, the hypothesis, that these superstructures were large spherical or worm-like micelles, was further supported by TEM. When applying even higher temperatures (above 50°C) as a second stimulus, smaller superstructures were found in the solutions of both types of copolymers. It can be assumed, that one of the two observed transitions is caused by the collapse of the PDEGMEMA block(s), and that the other one is related to the unfolding of the collagen-like helices. Moreover, analog di- and triblock copolymers based on POEGMEMA due to their higher LCST promise to be ideal candidates for comparative studies.

Acknowledgement

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the instrument. CD spectra and the TEM image were kindly provided by [redacted] from the group of [redacted] at the University of Delaware, Newark, USA.

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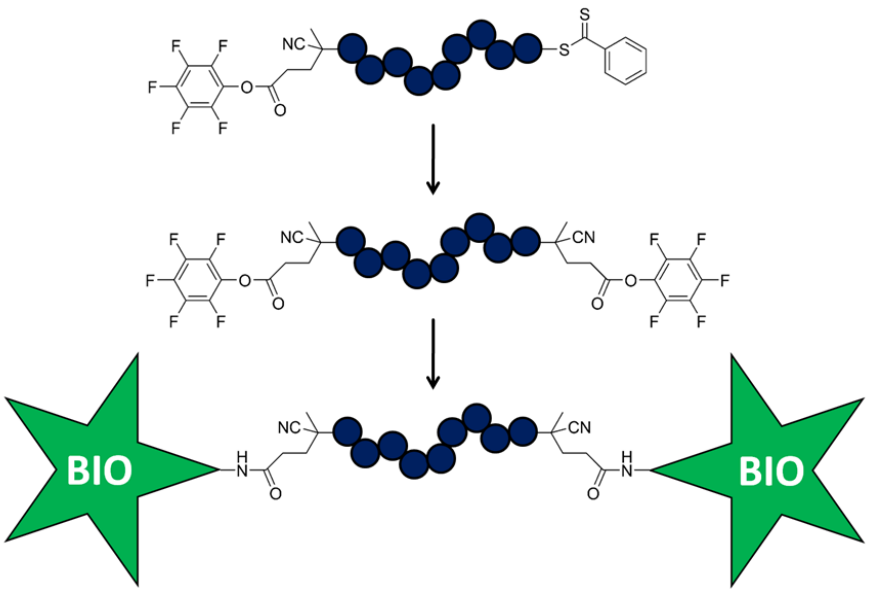
4.4 A Synthetic Route toward Peptide-Polymer-Peptide Triblock Copolymers via Bioconjugation



Introduction

With the help of recent advances in the development of controlled radical polymerization (CRP) techniques and the increasing number of precise end-group modification methods,[1-24] several synthetic strategies for site-selective conjugation of "smart" polymers to peptides and proteins have been proposed.[9,11,25-33] In the majority of the examples, linear polymer-*b*-peptide diblock copolymers were produced and it could be shown that these polymer-*b*-peptide diblock copolymers exhibit interesting material properties based on the combination of the complementary characteristics of the two combined material classes.[26,29,30,34-39] For instance, bioconjugates consisting of biologically active proteins and stimuli-responsive polymers were found to exhibit the same biological function as the unconjugated protein in terms of their bioactivity, however could be regulated by an external stimulus, such as a change in temperature, addressing the stimuli-responsive polymer block.[30,35-37] Other biohybrid systems self-assembled into superstructures on the nano- or even micrometer scale driven by the characteristic structure formation tendency of the peptide segment in

the hybrid, for example leading to silk-like nanofibers with good mechanical properties.[38,39-41]

However, only few examples demonstrating the synthesis of bioconjugates with more than one biological block at a defined position along the polymer chain can be found in literature.[31-33,42] In general, such structures could be obtained via conversion of synthetic polymers bearing two reactive chain ends with two peptides or proteins. Due to its high versatility in terms of possible end-group modifications,[1-18] reversible addition-fragmentation chain transfer (RAFT) polymerization is a highly suitable method for the preparation of such well-defined telechelic polymers allowing for the conjugation to at least two biological entities. An efficient tool for bioconjugation to the α-end-group of polymers obtained via RAFT polymerization, was reported by Theato and coworkers. A chain transfer agent (CTA) with an activated ester in the R-group was utilized resulting in well-defined polymers with an activated ester α-end-group, which allowed for conversion with the amine groups of different biomolecules, such as a collagen-like peptide and the hormone thyroxin.[8,9,42]

Herein, the expansion of this synthetic approach onto both polymer chain ends allowing the synthesis of symmetric peptide-*b*-polymer-*b*-peptide triblock copolymers is presented on the example of a telechelic, thermo-responsive poly(diethylene glycol methyl ether methacrylate) (PDEGMEMA). Hence, the potentially formed superstructures of these bioconjugates are very likely thermo-responsive structures themselves because of the PDEGMEMA block connecting the two peptide segments.

Moreover, the resulting bioconjugate holds high potential for further applications on the basis of the peptide segment. Bioconjugates consisting of similar building blocks, namely poly(ethylene glycol)-*b*-poly(aspartic acid) diblock copolymers, have shown to be suitable additives to gain control over the crystallization of $CaCO_3$ and $BaSO_4$[43] and could be used for drug delivery due to their ability to bind anticancer drugs such as adriamycin and to incorporate these into polymeric micelles.[44,45] Therefore, the chosen peptide, which is the aspartic acid-rich sequence $(Asp)_{15}$-$(Ser)_5$-Gly, is an

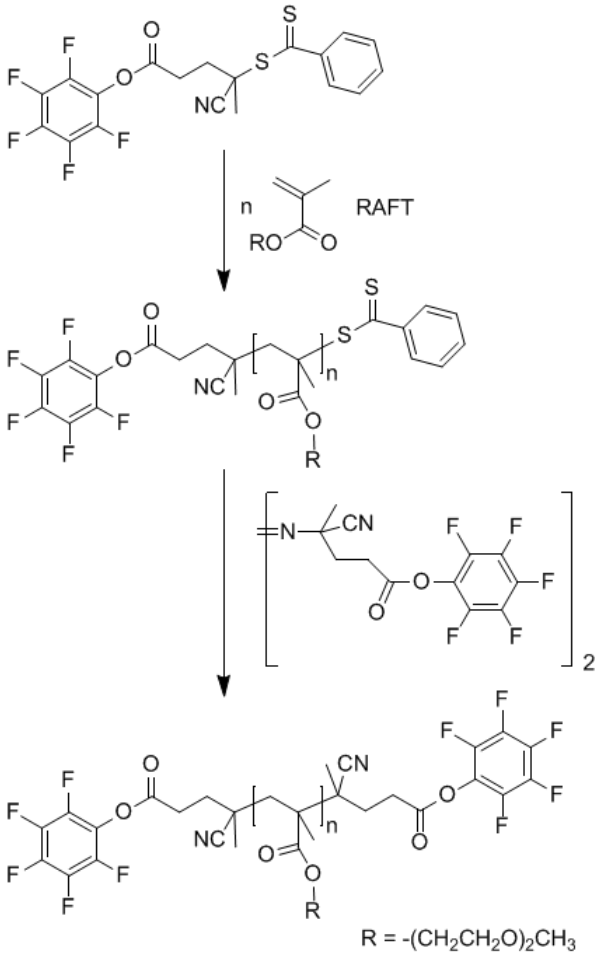
interesting candidate for the conjugation to PDEGMEMA, and it could be of further interest to study the influence of the triblock structure on the described applications in comparison to the diblock copolymers.

Results and Discussion

For the synthesis of a linear bioconjugate with two biological entities, a homotelechelic polymer precursor with two activated ester end-groups was prepared via RAFT polymerization using a CTA with a pentafluorophenyl ester in the R-group followed by radical substitution of the dithioester at the ω-end-group with a functional derivative of 2,2'-azobisisobutyronitrile (AIBN) for the introduction of a second pentafluorophenyl ester end-group. This functional derivative of AIBN, namely bis(pentafluorophenyl)azobis-(4-cyanovalerate) (PFP-ACV), resulted from the esterification of azobis(4-cyanovaleric acid) with pentafluorophenol, which was performed according to a previously published procedure with slight modifications.[8] Briefly, azobis(4-cyanovaleric acid) was reacted with pentafluorophenol in the presence of 2,6-lutidine and trifluoroacetic anhydride, so that intermediately, pentafluorophenyl trifluoroacetate was formed, which was the active species for the esterification. During the reaction as well as the isolation of the product being a thermal initiator, the temperature should not exceed 35°C in any step. The PFP-ACV was not only used later on for the modification of the ω-end-group, but was also a crucial reactant in the synthesis of the PFP-CTA required for the RAFT polymerization.[8] For this purpose, it was subjected to radical cross-coupling with dithiobenzoic acid disulfide under an inert gas atmosphere. The dark red product was purified via column chromatography.

RAFT polymerization of diethylene glycol methyl ether methacrylate (DEGMEMA) employing the PFP-CTA (**Scheme 1**) was performed following a standard procedure.[8,9] Monomer, CTA, and AIBN were dissolved in freshly distilled dioxane. This solution was degassed three times prior to polymerization, which was conducted at 70°C under argon atmosphere for 20 hours and yielded a pink, viscous

polymer after precipitation in hexane. Incorporation of the activated ester moiety was confirmed via ^{19}F NMR spectroscopy.



SCHEME 1: Synthetic pathway toward homotelechelic PDEGMEMA using the PFP-CTA and the PFP-ACV for end-group functionalization.

The dithioester ω-end-group of this PDEGMEMA was subsequently substituted by a pentafluorophenyl 4-cyanovalerate group (**Scheme 1**), which was introduced via treatment with an excess of the PFP-ACV in distilled dioxane at 80°C. The initially pink solution turned colorless during the course of the reaction, indicating the cleavage of the dithioester end-group, which was further confirmed via disappearance of its aromatic signals in ^{1}H NMR spectroscopy. After evaporation of most of the dioxane, the raw product was recovered by precipitation in diethyl ether, which was not a good solvent for the impurities neither. Predominantly, these insoluble impurities consisted of the recombination product of two radical

pentafluorophenyl 4-cyanovalerate fragments, which are formed after thermal cleavage of the diazo compound under liberation of elementary nitrogen. This side product was separated from the desired polymeric product via semi-preparative gel permeation chromatography (GPC) in tetrahydrofuran, after which ^{1}H NMR spectroscopy confirmed the abundance of the recombination product. The ^{19}F NMR spectrum of the final PDEGMEMA showed the three characteristic signals of the pentafluorophenyl ester, indicating the integrity of the ester linkages.

To verify the successful replacement of the dithioester moiety by a pentafluorophenyl 4-cyanovalerate ω-end-group more explicitly, the homotelechelic PDEGMEMA was characterized via matrix assisted laser desorption/ionization time of flight mass spectroscopy (MALDI TOF MS, see **Figure 1**).

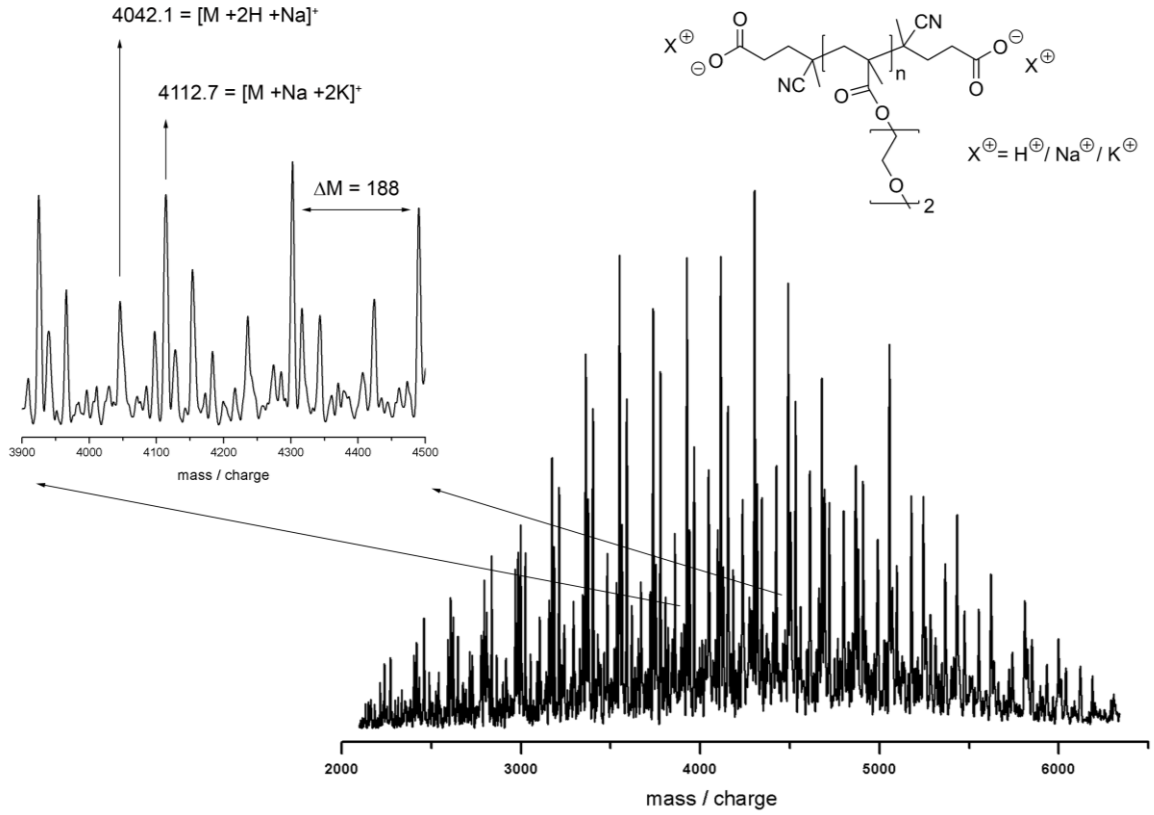


FIGURE 1: MALDI TOF mass spectrum of homotelechelic PDEGMEMA.

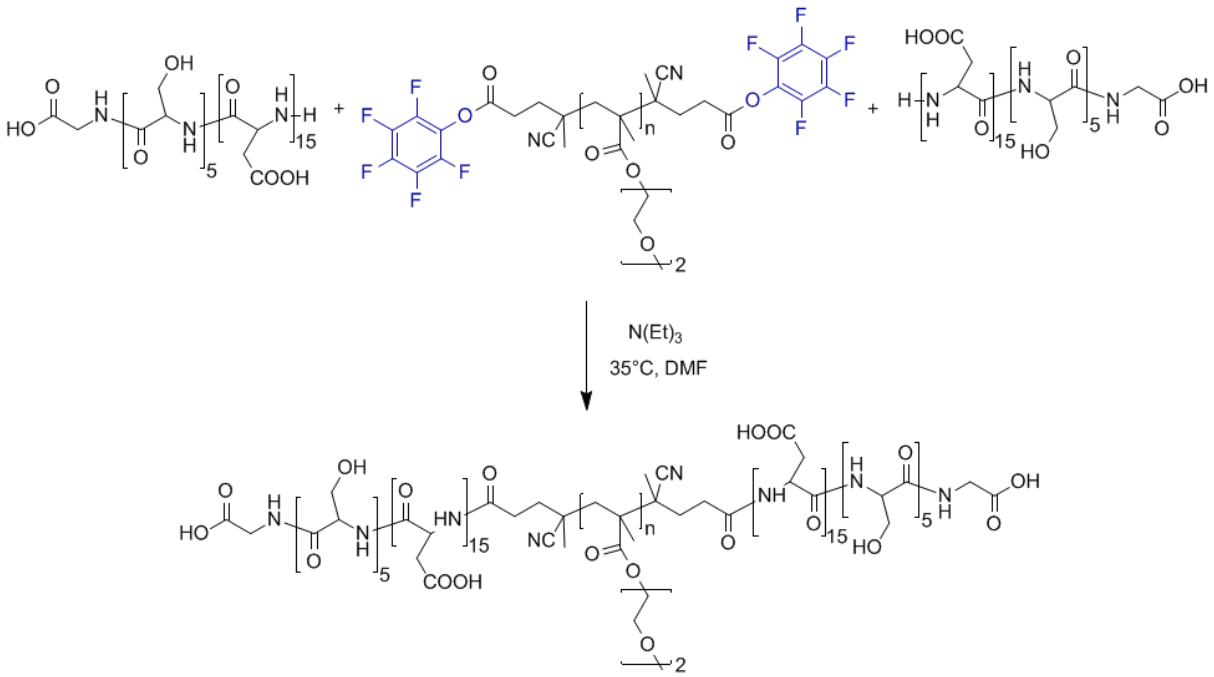
As expected, the detected molecular weight distribution exhibited an average mass difference between two peaks, ΔM, of 188 g/mol, corresponding to the molar mass of a DEGMEMA repeat unit. However, more than one molecular weight distribution

with this ΔM was found, and the absolute values of mass per charge, m/z, did not match the expected molecular weight of homotelechelic PDEGMEMA with two pentafluorophenyl ester end-groups. Nevertheless, the molecular weights detected could be assigned to a homotelechelic PDEGMEMA species with two 4-cyanovalerate end-groups, which can only be derived from the desired homotelechelic PDEGMEMA with two activated ester end-groups, and thus confirmed the successful substitution of the dithioester end-group by a pentafluorophenyl 4-cyanovalerate radical via the described procedure. It has to be assumed that potassium trifluoroacetate, which was used as for this sample indispensable cationization agent, cleaved the activated ester bonds in both end-groups, which were consequently observed as carboxylate end-groups in MALDI TOF MS. As counterions, a proton, sodium or potassium could be found, which explains the existence of more than one molecular weight distribution (two of the possible counterion permutations are shown exemplarily in **Figure 1**). In combination with the information obtained via ^{19}F NMR spectroscopy, these results confirmed the successful preparation of homotelechelic PDEGEMEMA with two activated ester end-groups.

With the reactive polymer precursor in hand, a peptide-*b*-polymer-*b*-peptide triblock copolymer could be synthesized via conversion of the described homotelechelic PDEGMEMA with the N-termini of two equivalents of an aspartic acid-rich peptide sequence, i.e. H_2N-$(Asp)_{15}$-$(Ser)_5$-Gly-OH. This peptide was produced via solid-phase peptide synthesis (SPPS) following a standard procedure for automated N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) SPPS. Afterward, it was cleaved from the resin and deproteced in one step using a mixture of dichloromethane and trifluoroacetic acid (1:1).

For bioconjugation to homotelechelic PDEGMEMA, four equivalents of the peptide were reacted with one equivalent of PDEGMEMA in dry *N*,*N*-dimethylformamide (**Scheme 2**). Triethylamine was added as auxiliary base to the initially turbid solution, which turned clear during the degassing, and was afterward stirred at 35°C

for two days under an argon atmosphere. The product could be precipitated in chloroform, which was a good solvent for PDEGMEMA, but not for the peptide. Hence, all the PDEGMEMA isolated via this method had to be conjugated to at least one peptide block in order to precipitate in chloroform. The solid, off-white product was characterized by ^{1}H and ^{19}F NMR spectroscopy, which confirmed that the isolated product contained PDEGMEMA as well as peptide, and that the activated ester end-groups were fully consumed during the reaction.



SCHEME 2: Synthesis of a peptide-b-PDEGMEMA-b-peptide triblock copolymer via activated ester chemistry.

Successful conjugation could be verified via GPC in DMF (+ 0.25M LiBr), which is displayed in **Figure 2**. For comparison, the elugram of the homotelechelic PDEGMEMA was plotted along with the elugram of the peptide-*b*-polymer-*b*-peptide triblock copolymer. However, the pure peptide was not well enough soluble in DMF to allow for a reproducible GPC measurement. The elugram of the product exhibited a relatively symmetrical higher molecular weight signal, no distinct signal at the retention time of the homopolymer, but weak, broadly distributed signals, most likely indicating residual peptide building block, which was used in excess for

the conjugation reaction and seemed to be soluble in DMF at least in small traces. Given that the PDEGMEMA building block was shown to exhibit two peptide-reactive end-groups, it was very unlikely, that pure PDEGMEMA-*b*-peptide diblock copolymer would be obtained from this conjugation experiment. Hence, the symmetry of the high molecular weight signal in the elugram of the product was a strong indication for a highly efficient double bioconjugation to the homotelechelic polymer precursor (an inefficient and only partly conversion of the two polymer chain ends with the peptide would result in mixture of di- and triblock copolymer causing an asymmetric or even bimodal signal in GPC). The polymer segment in the biohybrid presumably induced the better solubility of the bioconjugate in comparison to the pure peptide.

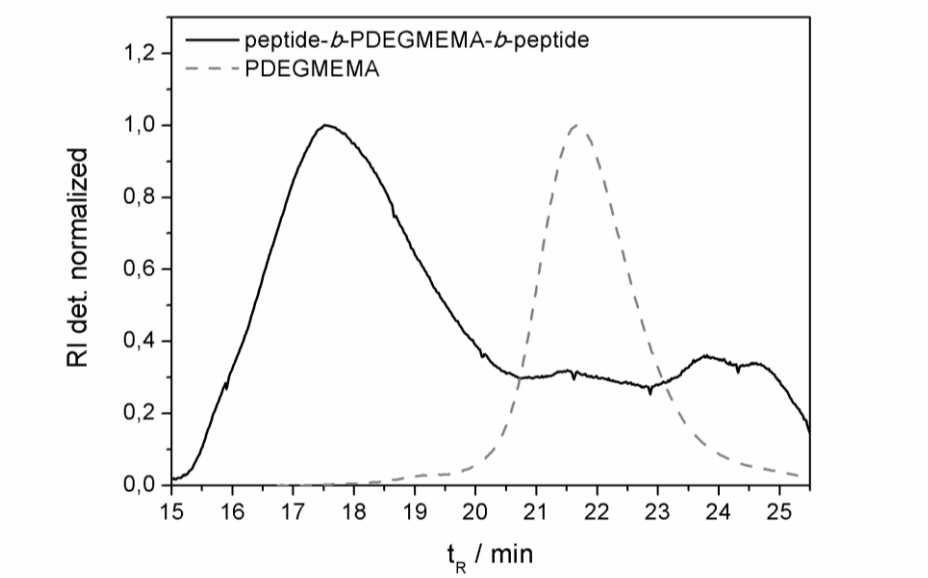


FIGURE 2: GPC elugram of the peptide-b-PDEGMEMA-b-peptide triblock copolymer in DMF (+ 0.25M LiBr) in comparison to the homopolymer.

The lower molecular weight signals assigned to small traces of dissolved residual peptide were irregularly distributed over a wide range of retention time, which was probably caused by interactions of the peptide with the column material, which were also described elsewhere in literature.[46] For separation of the residual peptide from the product, the raw product was dissolved in deionized water and subjected to dialysis in the same solvent for four days. However, the given time in aqueous might

have caused intermolecular aggregation of the peptide leading to an insolubility of the remaining product in DMF, so that no further GPC of the purified product could be recorded.

As one of the few techniques, which does not necessarily require good solubility of the sample, MALDI TOF MS was chosen to gain further information about the final product, even though desorption and ionization of such a high molecular weight species with heterogeneous structure can be challenging. This probably also explained the low intensities found in the MALDI TOF mass spectrum of the peptide-*b*-PDEGMEMA-*b*-peptide triblock copolymer. Nevertheless, the weak signals observed in this spectrum around 8000 g/mol exhibited an average distance of ΔM = 188 g/mol between the distinct peaks, which corresponded to the molecular weight of the DEGMEMA repeat unit. Moreover, these weak signals presumably represented the prevalent molecular weight species around the maximum of the molecular weight distribution of the triblock copolymer, and as such were observed in the expected size range for the described triblock copolymer.

In summary, the discussed measurements provided strong indication for an efficient conjugation of two peptide segments to the homotelechelic PDEGMEMA precursor via activated ester chemistry.

Experimental

Materials. All chemicals were commercially available and used as received unless mentioned otherwise. Diethylene glycol methyl ether methacrylate (DEGMEMA) was purified by distillation in vacuum. AIBN was recrystallized from diethyl ether. Tetrahydrofurane (THF) and dioxane were distilled from sodium / potassium, dichloromethane (for the synthesis of the PFP-ACV) from calcium hydride, and ethyl acetate from a mixture of potassium carbonate and sodium sulfate.

Instrumentation. ^{1}H-NMR spectra were recorded using a Bruker AC 300 MHz FT-NMR spectrometer working at 300 MHz, and ^{19}F-NMR spectra on a Bruker

376.5 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. For GPC in *N,N*-dimethylformamide (DMF) containing 0.25 M LiBr an Agilent 1100 Series GPC set-up with three PSS HEMA columns ($10^6/10^5/10^4$ g/mol), a UV and a RI detector was used, which was calibrated using PMMA standards by PSS (Polymer Standards Service, Mainz). The eluent was used at a flow rate of 1 mL/min and a temperature of 50°C. GPC in THF was performed on a GPC set-up consisting of the following components: a Jasco PU-1580 pump, a Jasco AS-1555 autosampler, MZ-Gel-SDplus columns (10^2, 10^4 and 10^6 Å^2), a Jasco UV-1575 UV/vis detector, and a Jasco RI-1530 refractive index detector. The system was calibrated using polystyrene standards by PSS. The semi-preparative GPC experiment was conducted on a KNAUER Smartline set-up consisting of a KNAUER pump 1000, a KNAUER UV detector 2500, a KNAUER RI detector 2400, and a MZ-Gel SD plus column (250 x 40 mm, 10^4 Å, 10 μm). The eluent (THF) was used at a flow rate of 25 mL/min.

Molecular weight distributions were measured on a Kratos Analytical Shimadzu AXIM-CFR MALDI TOF (matrix assisted laser desorption/ionization time of flight) mass spectrometer.

Synthesis of bis(pentafluorophenyl)azobis-(4-cyanovalerate) PFP-ACV. This functional derivative of AIBN was synthesized according to a previously published procedure[8] with slight modifications. In a dry round bottom flask with three necks equipped with a tap connected to a nitrogen balloon, a thermometer, and a dropping funnel, which was topped with a septum, the reactants azobis(4-cyanovaleric acid) (20 g, 71.35 mmol), pentafluorophenol (31.5 g, 171.23 mmol), and 2,6-lutidine (63 mL, 58.1 g, 542.24 mmol) are dissolved in dry dichloromethane (250 mL) under nitrogen and cooled down to 0°C. While the trifluoroacetic anhydride (30 mL, 45 g, 214.05 mmol) was added dropwise from the dropping funnel, the color of the suspension

turned ocher. This mixture was allowed to warm up to room temperature overnight, turned brown, and was then washed three times with saturated saline. The organic phase should have a neutral pH afterwards and was dried over magnesium sulfate. Most of the solvent was evaporated using a rotary evaporator and a temperature of maximum 35°C. In order to remove the remaining lutidine, the product was precipitated in cold hexane three times. After drying in vacuum, an off-white powder was obtained (26.3 g, 60%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.03 – 2.43 (m, 8H, CH_2); 1.78 and 1.73 (2s (cis, trans), 6H, CH_3); ^{13}C NMR ($CDCl_3$): δ (ppm) = 167.4 (O*C*(=O)); 142.6 (w, *C*F); 141.3 (w, *C*F); 139.3 (w, *C*F); 138.0 (w, *C*F); 136.2 (w, *C*F); 117.1 (*C*≡N); 71.7 (*C*C≡N); 32.6 (OC(=O)CH_2CH_2); 28.3 (OC(=O)CH_2); 23.8 (CH_3); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.42 (m, 2F); -157.08 (m, 1F); -162.76 (m, 2F).

Synthesis of pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA). The PFP-CTA was prepared via reaction of the PFP-ACV with dithiobenzoic acid disulfide according to a previously published procedure.[8] Briefly, in a dry round bottom flask with two necks equipped with a stir bar, a reflux condenser topped with a tap, and a septum, PFP-ACV (8.00 g, 13.06 mmol), dithiobenzoic acid disulfide (3.64 g, 11.88 mmol), and dry ethyl acetate (100 mL) were mixed and degassed. This solution was stirred at 80°C and under an inert gas atmosphere overnight. After evaporation of the solvent using a rotary evaporator, the raw product was dried in vacuum and then purified via column chromatography using a mixture of dichloromethane and petrolether (6:4) in a first run and a mixture of cyclohexane and chloroform (6:4) in a subsequent run. The solid red product was obtained after drying in vacuum (6.07 g, 57%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.92 (d, 2H, *J* = 7.9 Hz, *o*-Ar); 7.57 (t, 1H, *J* = 7.9 Hz, *p*-Ar); 7.40 (t, 2H, *J* = 7.9 Hz, *m*-Ar), 3.15 – 2.43 (m, 4H, CH_2CH_2); 1.98 (s, 3H, CH_3); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.91 (m, 2F); -157.67 (m, 1F); -162.25 (m, 2F).

Synthesis of PDEGMEMA with a pentafluorophenyl ester α-end-group. PDEGMEMA with a pentafluorophenyl ester α-end-group was obtained from a RAFT polymerization using the PFP-CTA following an already published standard procedure.[8,9] ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.83 (m, 2H, *o*-C*H* (phenyl end-group)); 7.48 (m, 1H, *p*-C*H* (phenyl end-group)); 7.32 (m, 2H, *m*-C*H* (phenyl end-group)); 4.08 (br, 2nH, C(=O)OCH_2CH$_2$); 3.66 (br, 2nH, C(=O)OCH$_2$CH_2); 3.60 (br, 2nH, CH_2CH$_2$OCH$_3$); 3.54 (br, 2nH, CH$_2$CH_2OCH$_3$); 3.37 (br, 3nH, CH$_2$CH$_2$OCH_3); 2.85 (w, 2H, C(=O)CH_2 (end-group)); 2.15 – 1.61 (br, 2nH, CH_2 (backbone)); 1.52 – 1.20 (w, 5H, C(=O)CH$_2$CH_2(CH_3) (end-group)); 1.15 – 0.70 (br, 3nH, CCH_3 (backbone)); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.59 (m, 2F); -157.48 (m, 1F); -161.97 (m, 2F); GPC (THF): M_n = 2600 g/mol; M_w = 2900 g/mol; PDI = 1.10.

Conversion of the ω-end-group of PDEGMEMA with PFP-ACV. In a dry round bottom flask with a stir bar, a septum and an argon balloon, PDEGMEMA (1 g, $3.85*10^{-4}$ mol) and PFP-ACV (4.8 g, $7.69*10^{-3}$ g/mol, 20 equivalents) were dissolved in freshly distilled dioxane (100 mL). The initially pink solution was stirred at 80°C for 2 hours and then at room temperature overnight, before the solvent of the now almost colorless solution was evaporated almost completely with a rotary evaporater. The remaining raw product was diluted with THF and precipitated in diethyl ether. Subsequently, the product was purified via semi-preparative GPC (THF). After evaporating the THF and drying in vacuum, the slightly yellowish, viscous product was obtained (900 mg, 90%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.08 (br, 2nH, C(=O)OCH_2CH$_2$); 3.66 (br, 2nH, C(=O)OCH$_2$CH_2); 3.60 (br, 2nH, CH_2CH$_2$OCH$_3$); 3.54 (br, 2nH, CH$_2$CH_2OCH$_3$); 3.37 (br, 3nH, CH$_2$CH$_2$OCH_3); 2.85 (w, 2H, C(=O)CH_2 (end-group)); 2.13 – 1.60 (br, 2nH, CH_2 (backbone)); 1.54 – 1.17 (w, 5H, C(=O)CH$_2$CH_2(CH_3) (end-group)); 1.17 – 0.70 (br, 3nH, CCH_3 (backbone)); ^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -152.58 (m, 4F); -157.50 (m, 2F); -162.00 (m, 4F); GPC (THF): M_n = 2600 g/mol; M_w = 2800 g/mol; PDI = 1.10; GPC (DMF): M_n = 2500 g/mol; M_w = 3000 g/mol; PDI = 1.20; MALDI TOF MS: The matrix (dithranole) and the

sample were independently dissolved in chloroform (10 mg/mL), while the cationization agent, namely KTFA, was dissolved in methanol (1 mg/mL). 2 µL of each of these solutions were placed onto a multistage target plate independently (1st matrix, 2nd sample, 3rd salt), allowing each solution to dry, before the next solution was added on top. The results discussed below were obtained in linear detection mode. MALDI TOF MS (*m*/*z*): calcd. for $[C_6H_7NO_2(C_9H_{16}O_4)_{20}C_6H_7NO_2]^{2-}$: 4015.3; found: 4112.7 $[M +Na +2K]^+$; 4042.1 $[M +2H +Na]^+$.

Synthesis of the peptide H-(Asp)$_{15}$-(Ser)$_5$-Gly-OH. The peptide segment was synthesized via solid-phase peptide synthesis (SPPS) following a standard procedure for automated N^{α}-9-fluorenylmethoxycarbonyl (Fmoc) SPPS. A 2-chlorotrityl chloride resin preloaded with Fmoc-glycine was used. Cleavage from the resin and deprotection of the amino acid residues were performed in one step using a mixture of dichloromethane and trifluoroacetic acid, so that the deprotected peptide segment should be obtained. ^{1}H NMR (D_2O): δ (ppm) = 5.35 – 4.25 (br, 22H, $C_{\alpha}H$); 3.85 (br, 10H, $C_{\alpha}HCH_2OH$); 3.06 – 2.62 (br, 30H, $C_{\alpha}HCH_2C(=O)OH$); 1.69 – 1.32 (w, br, 9xH, $C(CH_3)_3$ small amount of residual *t*-Bu).

Synthese of a peptide-*b*-PDEGMEMA-*b*-peptide triblock copolymer. In a small reaction tube equipped with a stir bar, homotelechelic PDEGMEMA (25 mg, 9.6*10^{-6} mol, 1 equivalent), the unprotected peptide sequence H_2N-(Asp)$_{15}$-(Ser)$_5$-Gly (82.9 mg, 3.7*10^{-5} mol, 4 equivalents), and triethylamine (roughly 5 µl, but at least 2.6 µL, 1.86*10^{-5} mol, 2 equivalents) were dissolved in dry DMF (2 mL). After degassing the initially turbid solution for 2 minutes using a nitrogen stream, the solution turned clear, and the reaction tube was capped with septum and an argon balloon afterwards. The reaction was stirred at 35°C for 2 days, before the product was precipitated in chloroform. After drying in vacuum, the solid off-white product was obtained (38.8 mg, 5.7*10^{-6} mol, 61%). ^{1}H NMR (DMSO): δ (ppm) = 8.75 – 7.55 (br, 42H, N*H*); 4.91 (w, 4H, $C_{\alpha}H_2$); 4.46 (br, 30H, $C_{\alpha}H$ (Asp)); 4.28 (br, 10H, $C_{\alpha}H$ (Ser)); 3.97

(br, 2nH, C(=O)OC*H*$_2$CH$_2$); 3.80 – 3.27 (br, 6nH + 20H, C*H*$_2$OC*H*$_2$C*H*$_2$OCH$_3$ and C$_\alpha$HC*H*$_2$OH); 3.22 (br, 3nH, CH$_2$CH$_2$OC*H*$_3$); 2.80 – 2.25 (br, 60H, C$_\alpha$HC*H*$_2$C(=O)OH); 2.00 – 1.60 (w, br, 2nH, C*H*$_2$ (backbone)); 1.60 – 1.23 (w, br, 9xH, C(C*H*$_3$)$_3$); 1.00 – 0.62 (w, br, 1nH, CC*H*$_3$ (backbone)); ^{19}F NMR (376 MHz, CDCl$_3$): no residual signals; GPC (DMF): M_n = 23400 g/mol; M_w = 28300 g/mol; PDI = 1.54. The product was further purified via dialysis in deionized water using a Spectra/Por® regenerated cellulose membrane with a molecular weight cut-off of 4000-6000 g/mol for 4 days. The solid white product was obtained after lyophilization.

MALDI TOF MS: The matrix (α-cyano-4-hydroxycinnamic acid, CHCA) and the sample were independently dissolved in methanol and chloroform, respectively (10 mg/mL). 2 μL of each of these solutions were placed onto a multistage target plate independently (1st matrix, 2nd sample), allowing each solution to dry, before the next solution was added on top. The results discussed below were obtained in linear detection mode. MALDI TOF MS (*m*/*z*): calcd. for [C$_2$H$_3$NO$_2$(C$_3$H$_5$NO$_2$)$_5$(C$_4$H$_4$NO$_3$)$_{15}$C$_6$H$_7$NO(C$_9$H$_{16}$O$_4$)$_{12}$C$_6$H$_7$NO(C$_4$H$_4$NO$_3$)$_{15}$(C$_3$H$_5$NO$_2$)$_5$ C$_2$H$_3$NO$_2$]$^{32-}$ 33X$^+$ = Gly-Ser$_5$-Asp$_{15}$-linker-(DEGMEMA)$_{12}$-linker-Asp$_{15}$-Ser$_5$-Gly + 33X$^+$ (X = Na$^+$/K$^+$): 6917 + 33X$^+$; found: e.g. 7531.2, 7710.7, 7915.1, 8117.0, 8300.3.

Conclusion

Via combination of a controlled radical polymerization mediated by a functional chain transfer agent, namely the PFP-CTA, with a radical substitution of the resulting dithioester ω-end-group using a functional derivative of AIBN, namely the PFP-ACV, homotelechelic PDEGMEMA was obtained with two activated ester end-groups. The homotelechelic structure was confirmed via MALDI TOF MS in combination with ^{1}H and ^{19}F NMR spectroscopy. This doubly peptide-reactive polymer precursor was converted with the N-termini of two equivalents of an aspartic acid-rich peptide yielding a peptide-*b*-PDEGMEMA-*b*-peptide triblock copolymer. Even though the analysis of this bioconjugate was complicated by the solubility behavior of the peptide segment, the combination of NMR spectroscopy,

GPC, and MALDI TOF MS led us to the conclusion that the conversion of both activated ester end-groups of the homotelechelic PDEGMEMA was successful. In summary, a generally applicable synthetic route toward well-defined homotelechelic polymers with two pentafluorophenyl ester end-groups and its application for multivalent bioconjugation was demonstrated on the example of PDEGMEMA.

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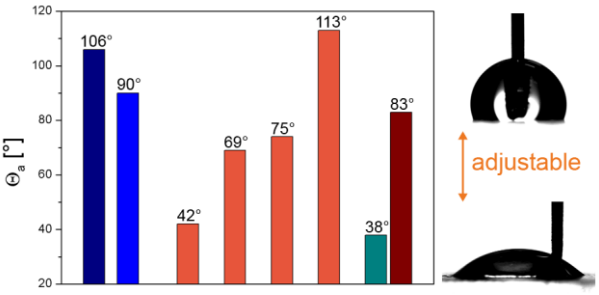
4.5 Versatile Responsive Surfaces via Hybrid Polymers Containing Acetal Side Groups

Wiss, K. T.; Kessler, D.; Wendorff, T. J.; Theato, P. *Macromol. Chem. Phys.* **2009**, *210*, 1201-1209.



Abstract

Two hybrid polymers, poly(methylsilsesquioxane)-poly(2,2-diethoxyethyl acrylate) and poly(methylsilsesquioxane)-poly(1,3-dioxolan-2-ylmethyl acrylate), were synthesized and used for preparation of stable surface coatings. Their acetal side groups could be functionalized via solution-dipping with different primary amines and hydroxylamines after acidic deprotection and thus allowed for flexible modification of the surface hydrophilicity. Functionalization with a thermo-responsive polymer resulted in a thermo-responsive surface with temperature-dependent contact angles. Further, the two types of acetals showed different stability towards acidic treatment and could be addressed independently.

Introduction

Functional materials for the design of versatile responsive surfaces have gained not only interest in science but are also used in a large number of applications in everyday life, for example protective coatings on bottles, scratch-resistant coatings on glass or CDs and DVDs, or non-stick coatings in pans, as well as high-end products,

for example microfluidic devices for "lab-on-a-chip" systems for medical diagnostics and more.[1-3] Considering the wide variety of possible applications, it becomes obvious that a universal coating material needs to be adherent to lots of different substrate materials (glass, metal, plastics…) while fulfilling diverse functions. Thus, the two main challenges for the design of such a coating material are film stabilization on different substrates and simple modification in order to functionalize the resulting films. Silica and especially bio-inspired silica based materials have been recognized as an interesting and potential class of materials.[4-7] In this regard, Theato and coworkers have investigated the synthesis of inorganic/organic hybrid materials based on poly(methylsilsesquioxane) (PMSSQ) and poly(acrylates) via reversible addition-fragmentation chain transfer (RAFT) polymerization and their use as coating materials.[8] After film preparation of these hybrid polymers via spin-coating, stable films were realized on glass, metal as well as plastic substrates, due to their remaining ability to be thermally cross-linked. Furthermore, the use of pentafluorophenyl acrylate as monomer for the organic block enabled the easy functionalization of these surface coatings with primary amines whose residual groups determine the resulting surface properties.[9] For example, this system was successfully used to tune the hydrophilicity of the coated surface. However, for certain applications, it would be useful to have the possibility to introduce several functionalities onto one surface. Therefore, surface coatings with orthogonally reactive functional groups are desirable. Functional groups that could be converted independently of activated ester groups,[10,11] are acetal groups as protected aldehyde groups, which can be functionalized after acidic deprotection.[12-16] Within the present study, we investigate films prepared from the copolymers poly(methylsilsesquioxane)-poly(2,2-diethoxyethyl acrylate) (PMSSQ-PDEEA) and poly(methylsilsesquioxane)-poly(1,3-dioxolan-2-ylmethyl acrylate) (PMSSQ-PDMA). These copolymers contained diethyl acetal side groups and cyclic ethylene acetal side groups, respectively, which are expected to exhibit different stability towards acidic deprotection and thus to be functionalized under different conditions.[17]

Experimental Part

Reagents All chemicals and solvents were commercially available and used as received unless otherwise indicated. Tetrahydrofuran (THF) and dioxane were distilled from a mixture of sodium and potassium under nitrogen. 2,2'-Azobis(2-methylpropionitrile) (AIBN) was recrystallized from diethyl ether. Di(ethylene glycol) methyl ether methacrylate (DEGMEMA) was purified by distillation in vacuum.

Instrumentation Gel permeation chromatography (GPC) in THF (sample concentration: 2.5 mg/mL) was used to determine molecular weights and molecular weight distributions, M_w/M_n, of polymer samples with respect to polystyrene standards (PSS). Therefore, a GPC set-up was used consisting of the following compounds: a Jasco PU-1580 pump, a Jasco AS-1555 autosampler, MZ-Gel-SDplus columns (10^2, 10^4 and 10^6 Å^2), a Jasco RI-1530 refractive index detector, a Jasco UV-1575 UV/vis detector and a Mini Dawn light scattering by Wyatt Technology Corporation. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AC 300 MHz FT-NMR spectrometer. ^{29}Si solid state NMR measurements were performed on a Bruker DRX 400 MHz FT-NMR spectrometer (rotation: 5000 Hz, T = RT, 4 mm rotor). Infrared (IR) spectra were obtained on a Nicolet 5 DXC FT-IR spectrometer with a Specac ATR unit and were uncorrected. A Veeco Dimension 3100 microscope was used for atomic force microscopy (AFM).

Synthesis of 2,2-diethoxyethyl acrylate (DEEA) This monomer was synthesized according to the procedure published by Zábranský et al.[18] Briefly, 16.65 g (0.18 mol) sodium acrylate, 29.56 mL (0.19 mol) 2-bromo-1,1-diethoxyethane, 0.2 g (1.6 mmol) *p*-methoxyphenol and 0.22 g (0.80 mmol) tetrabutyl ammonium chloride were heated to 160°C for 2 hours in 100 mL absolute dimethylformamide (DMF) and under nitrogen. After filtration, the liquid colorless product was isolated by distillation in vacuum (6.15 g, 33 mmol, 18%). Boiling point = 80°C at 20 mbar. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.42 (d, 1H, CH*H*=CH, ^{3}J = 17.3 Hz); 6.13 (dd, 1H, CHH=C*H*, $^3J_1$ = 17.3 Hz, $^3J_2$ = 9.9 Hz); 5.83 (d, 1H, C*H*H=CH, ^{3}J = 9.9 Hz); 4.70 (t, 1H, C*H*$(OEt)_2$, ^{3}J = 5.4 Hz); 4.16

(d, 2H, C(O)OC*H*$_2$, ^{3}J = 5.4 Hz); 3.68 (m, 2H, CH*H*-CH$_3$); 3.56 (m, 2H, C*H*H-CH$_3$); 1.20 (t, 6H, CH$_2$-C*H*$_3$, ^{3}J = 6.3 Hz); FT-IR (ATR): 3083 (w) (H$_2$C=); 2978; 2935; 2884; 1730 (C=O); 1636 (C=C); 1406; 1193; 1132; 1063 (C-O-C); 985 cm^{-1} (H$_2$C=).

Synthesis of 1,3-dioxolan-2-ylmethyl acrylate (DMA) For the synthesis of this monomer, the procedure by Zábranský et al.[18] was modified slightly with respect to the initial ratio of the reactants. 19.82 g (0.21 mol) sodium acrylate, 12 mL (0.12 mol) 2-bromomethyl-1,3-dioxolane, 0.43 g (3.46 mmol) *p*-methoxyphenol and 0.27 g (0.97 mmol) tetrabutyl ammonium chloride were heated to 135°C for 4 hours in absolute DMF and under nitrogen. After filtration, the liquid colorless product was isolated by distillation in vacuum (3.70 g, 23.4 mmol, 20%). Boiling point = 90°C at 20 mbar. ^{1}H NMR (CDCl$_3$): δ (ppm) = 6.43 (d, 1H, CH*H*=CH, ^{3}J = 17.3 Hz); 6.14 (dd, 1H, CHH=C*H*, ^{3}J$_1$ = 17.3 Hz, ^{3}J$_2$ = 10.7 Hz); 5.84 (d, 1H, C*H*H=CH, ^{3}J = 10.7 Hz); 5.16 (t, 1H, C*H*(OR)$_2$, ^{3}J = 3.5 Hz); 4.19 (d, 2H, C(O)OC*H*$_2$, ^{3}J = 3.5 Hz); 4.00 (m, 2H, C*H*H-C*H*H); 3.90 (m, 2H, CH*H*-CH*H*); FT-IR (ATR): 3106 (w) (H$_2$C=); 2985; 2960; 2889; 1726 (C=O); 1635 (C=C); 1408; 1189; 1147; 1067; 1042 (C-O-C); 985, 954 cm^{-1} (H$_2$C=).

Synthesis of the PMSSQ-RAFT agent The PMSSQ-RAFT agent was synthesized as published previously.[8,19] ^{1}H NMR (THF-d$_8$): δ (ppm) = 7.99 (br, 5H, phenyl); 7.36 (br, 2H, phenylen); 7.20 (br, 2H, phenylen); 5.80 (br, 1.9H, O*H*); 4.55 (br, 2H, SC*H*$_2$); 3.48 (br, 1.1H, OC*H*$_3$); 2.71 (br, 2H, SiCH$_2$C*H*$_2$); 0.99 (br, 2H, SiC*H*$_2$CH$_2$); 0.17 (br, 69.1H, SiC*H*$_3$); ^{29}Si solid state NMR: δ (ppm) = -48.27; -57.41; -66.47; GPC: M$_n$ = 5000 g/mol, PDI = 1.63.

Synthesis of PMSSQ-PDEEA[19] 0.5 g (0.1 mmol) of the PMSSQ-RAFT agent, 20 mg (0.12 mmol) AIBN, 3 mL dioxane and 2 g (10.6 mmol) DEEA were placed in a Schlenk flask, degassed and stirred for 4 hours at 80°C. The pink polymer was precipitated and reprecipitated in cold *n*-hexane and dried in high vacuum (yield: 2.23 g, 89%). ^{1}H NMR (CDCl$_3$): δ (ppm) = 4.64 (br, 1H, C*H*(OEt)$_2$); 4.02 (br, 2H, C(O)OC*H*$_2$); 3.64 (br m, 2H, CH*H*-CH$_3$); 3.52 (br m, 2H, C*H*H-CH$_3$); 2.37 (br, 1H, C*H* backbone); 2.05 – 1.35 (br, 2H, C*H*$_2$ backbone); 1.17 (br t, 6H, CH$_2$-C*H*$_3$); 0.13 (br, 3H, Si-C*H*$_3$); GPC: M$_n$ = 30100 g/mol, M$_w$ = 42000 g/mol, PDI = 1.39. FT-IR (ATR, cured

coating on glass): 2972, 2922, 2879 (CH_2 and CH_3, shape typical of dietyl acetal); 1738 (C=O); 904, 760, 410 cm^{-1} (br, Si-O).

Synthesis of PMSSQ-PDMA 0.5 g (0.1 mmol) of the PMSSQ-RAFT agent, 20 mg (0.12 mmol) AIBN, 3 mL dioxane and 2 g (12.6 mmol) DMA were placed in a Schlenk flask, degassed and stirred for 4 hours at 80°C. The pink polymer was precipitated and reprecipitated in cold *n*-hexane and dried in high vacuum (yield: 2.38 g, 95%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 5.08 (br, 1H, C*H*$(OR)_2$); 4.07 (br, 2H, C(O)OC*H*$_2$); 3.95 (br, 2H, C*H*H-C*H*H); 3.84 (br, 2H, CH*H*-CH*H*); 2.39 (br, 1H, C*H* backbone); 2.05 – 1.35 (br, 2H, C*H*$_2$ backbone); 0.13 (br, 3H, Si-C*H*$_3$); GPC: M_n = 37100 g/mol, M_w = 59600 g/mol, PDI = 1.61. FT-IR (ATR, cured coating on glass): 2950, 2885 (CH_2); 1734 (C=O); 891, 760, 410 cm^{-1} (br, Si-O).

Synthesis of PEG amine (M ~ 550 g/mol), PEGA, and di(ethylene glycol) methyl ether amine, DEGA The poly/di(ethylene glycols) were synthesized according to a protocol published by Mongondry et al.[20] with slight modifications. Phthalimide was alkylated in a Mitsunobu reaction using the respective PEG/DEG alcohol, and hydrozinolysis of the resulting phthalimido-PEG/DEG gave the amino-terminated compounds (yield: PEGA 86%, colorless viscous oil; DEGA 12%, colorless viscous liquid). For the synthesis of PEGA, the described procedure was followed exactly except for the reaction time in the second reaction step. Here, the phthalimido-PEG was reacted with the hydrazine for 5 hours at 85°C first and then for 12 hours at room temperature and the reaction yield was 90%. Phthalimido-PEG: ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.79-7.87 (m, 2H, 2/2'-phenyl); 7.66-7.74 (m, 2H, 3/3'-phenyl); 3.87 (t, 2H, NC*H*$_2$, ^3J = 6.2 Hz); 3.46-3.81 (m, (4n+2)H, OC*H*$_2$); 3.55 (s, 3H, OC*H*$_3$).

The DEG compounds were purified according to a modified procedure in both reaction steps and the reaction times were varied. After the reaction of DEG with pthalimide (2 days under cooling with a water bath), ethanol was added to the mixture and the solvents were removed under reduced pressure. The oily product was dissolved in a mixture of petrol ether and ethyl acetate (1:1) and stirred for 1 hour at 40°C. The precipitating solid was removed by filtration and the solvents were

evaporated. The resulting raw product was directly used for the second reaction step without further characterization. It was reacted with the hydrazine for 15 hours under reflux, then concentrated hydrochloric acid was added to reach a pH of 2-3. This mixture was heated under reflux for 1 hour, before the precipitating salt could be removed by filtration and the solvent could be evaporated. The raw product was dissolved in deionized water and an aqueous solution of sodium hydroxide was added to reach a pH of 11. The product was extracted with dichloromethane, the organic layers were dried with $MgSO_4$ and the solvent was evaporated. From the obtained colorless liquid, the precipitating salt was removed by filtration and the product was purified by distillation under high vacuum. PEGA: ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.40 (br, 2H, N*H*$_2$); 3.47-3.88 (m, (4n)H, (OC*H*$_2$C*H*$_2$)$_n$); 3.47-3.54 (m, 2H, C*H*$_2$CH$_2$NH$_2$); 3.35 (s, 3H, OC*H*$_3$); 2.93-3.08 (m, 2H, CH$_2$C*H*$_2$NH$_2$). DEGA: ^{1}H NMR ($CDCl_3$): δ (ppm) = 3.56-3.43 (m, 6H, OC*H*$_2$); 3.33 (s, 3H, C*H*$_3$); 2.81 (t, 2H, C*H*$_2$NH$_2$, ^{3}J = 6.1 Hz); 1.39 (s, 2H, N*H*$_2$).

Synthesis of amino-terminated poly(di(ethylene glycol) methyl ether methacrylate) (PDEGMEMA) DEGMEMA was polymerized via RAFT polymerization using pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA) as chain transfer agent according to the procedure published by Roth et al.[21] Briefly, 1.5 g (7.97 mmol) DEGMEMA, 177 mg (0.40 mmol) PFP-CTA, 6.5 mg (0.04 mmol) AIBN and 3 mL of freshly distilled dioxane were combined in a Schlenk flask equipped with stir bar. The solution was degassed by three freeze-pump-thaw cycles and the flask was refilled with argon. For the polymerization, the flask was placed into a preheated and stirred oil bath set to 70°C for 20 hours. The dark red viscous product was isolated by threefold precipitation into cold hexane and dried in vacuum (yield 1.23 g, 82%). ^{1}H NMR ($CDCl_3$): δ (ppm) = 7.86 (m, 2H, phenyl (end-group)); 7.50 (m, 1H, phenyl (end-group)); 7.34 (m, 2H, phenyl (end-group)); 4.08 (br, 2H, C(O)OC*H*$_2$CH$_2$); 3.66 (br, 2H, C(O)OCH$_2$C*H*$_2$); 3.60 (br, 2H, C*H*$_2$CH$_2$OCH$_3$); 3.54 (br, 2H, CH$_2$C*H*$_2$OCH$_3$); 3.37 (br, 3H, CH$_2$CH$_2$OC*H*$_3$); 2.40 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.10 – 1.70 (br, 2H, CH$_2$ backbone); 1.50-1.10 (w, 5H, C(=O)CH$_2$C*H*$_2$(C*H*$_3$)

(end-group)); 1.10 – 0.75 (br, 3H, CH_3 backbone); ^{19}F NMR ($CDCl_3$): δ (ppm) = -153.09 (m, 2F); -158.09 (m, 1F); -162.56 (m, 2F); GPC: M_n = 5500 g/mol, PDI = 1.10.

In the second step, 1.2 g (0.22 mmol) PDEGMEMA and 20 equivalents of AIBN (0.72 g, 4.4 mmol) were dissolved in 45 mL dry dioxane for the substitution of the dithioester end-group[22] resulting from the RAFT polymerization. The reaction mixture was heated to 80°C for 2.5 hours and the resulting yellowish viscous polymer was precipitated three times into a cold mixture of hexane and diethyl ether (50:50) and dried in vacuum. The ^{1}H NMR spectrum showed no remaining residues of AIBN, the replacement of the phenyldithioester (disappearance of the aromatic signals) and the reaction was almost quantitative. The ^{19}F NMR showed the three characteristic peaks of the pentafluorphenylester end-group. ^{1}H NMR ($CDCl_3$): δ (ppm) = 4.08 (br, 2H, C(O)OC*H*$_2$CH$_2$); 3.66 (br, 2H, C(O)OCH$_2$C*H*$_2$); 3.60 (br, 2H, C*H*$_2$CH$_2$OCH$_3$); 3.54 (br, 2H, CH$_2$C*H*$_2$OCH$_3$); 3.37 (br, 3H, CH$_2$CH$_2$OC*H*$_3$); 2.40 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.10 – 1.70 (br, 2H, CH_2 backbone); 1.50-1.10 (w, 11H, C(=O)CH$_2$C*H*$_2$(C*H*$_3$) and C(C*H*$_3$)$_2$CN (end-groups)); 1.10 – 0.75 (br, 3H, CH_3 backbone); ^{19}F NMR ($CDCl_3$): δ (ppm) = -153.09 (m, 2F); -158.09 (m, 1F); -162.56 (m, 2F); GPC: M_n = 5500 g/mol, PDI = 1.15.

For the conversion of the pentafluorophenylester end-group into an amino group, the polymer (220 mg, 0.04 mmol) is dissolved in 4 mL THF, 50fold excess of ethylene diamine (134 µL, 2 mmol) is added quickly and the solution is stirred overnight at 40°C. After evaporation of half of the solvent, the yellowish product is precipitated three times into a cold mixture of hexane and diethyl ether (50:50) and dried in vacuum (180 mg, 33 µmol, 82%). The ^{19}F NMR, measured at a comparable concentration as before the conversion, did not show any ^{19}F signals and thus indicated full conversion of the pentafluorophenylester end-group. ^{1}H NMR ($CDCl_3$): δ (ppm) = 6.41 (w, 3H, N*H*$_2$ and N*H*); 4.08 (br, 2H, C(O)OC*H*$_2$CH$_2$); 3.66 (br, 2H, C(O)OCH$_2$C*H*$_2$); 3.60 (br, 2H, C*H*$_2$CH$_2$OCH$_3$); 3.54 (br, 2H, CH$_2$C*H*$_2$OCH$_3$); 3.37 (br, 3H, CH$_2$CH$_2$OC*H*$_3$); 2.78 (m, 2H, C*H*$_2$NHC(=O) (end-group)), 2.30 (m, 2H, C*H*$_2$NH$_2$ (end-group)); 2.13 (w, 2H, C(=O)C*H*$_2$ (end-group)); 2.10 – 1.70 (br, 2H, CH_2

backbone); 1.50-1.10 (w, 11H, C(=O)$CH_2CH_2(CH_3)$ and C(CH_3)CN (end-groups)); 1.10 – 0.75 (br, 3H, CH_3 backbone).

Film preparation Polymer films were prepared via spin-coating of a 10 wt.-% solution in THF on a previously cleaned glass substrate (spin-coating speed: 4000 rpm; time: 15 s). Afterwards, the films were cured at 130°C for 1 hour. For the AFM measurements, the samples were prepared on cleaned silicon wafers.

Deprotection of PMSSQ-PDEEA films For the deprotection of the aldehyde groups in the PMSSQ-PDEEA films, the coated substrates were placed into a mixture of trifluoroacetic acid (TFA) and deionized water (2:1) for 1 hour at room temperature. After the reaction, the surface was washed three times with dionized water to remove the excess of TFA and then washed once with THF and dried with a nitrogen stream. FT-IR (ATR, coating on glass): 1738 (C=O); 902, 760, 410 cm^{-1} (br, Si-O).

Deprotection of PMSSQ-PDMA films For the deprotection of the aldehyde groups in the PMSSQ-PDMA films, the coated substrates were placed into a mixture of TFA and deionized water (8:1) for 1 hour at room temperature. After the reaction, the surface was washed three times with dionized water to remove the excess of TFA and then washed once with THF and dried with a nitrogen stream. FT-IR (ATR, coating on glass): 2980-2800 (br); 1734 (C=O); 891, 760, 410 cm^{-1} (br, Si-O).

Functionalization of PMSSQ-PDEEA films with amines After the deprotection, the free aldehyde groups of the films were converted with different amines. Generally, the substrates were placed into a 10wt.-% solution of the amine in THF for 1 hour at room temperature and after the functionalization, the excess of amine is removed by washing the surface twice with THF prior to drying the films with a nitrogen stream. Depending on the amine, the conversion was monitored via IR measurements and/or measurements of the advancing contact angle of water drops on the coated surfaces.

Functionalization of a PMSSQ-PDEEA film with amino-terminated polymers (PEG and PDEGMEMA) For the conversion of the deprotected aldehyde groups of the coating with the amino-terminated polymers, the procedure was the same as for the conversion with the small amines except that a longer reaction time (2 hours) was

chosen in order to allow the amino end-group of the polymers to react completely with the surface.

Synthesis of *O*-heptylhydroxylamine (HA1) and *O*-(3-hydroxypropyl)hydroxylamine (HA2) The hydroxyl amines were synthesized following the procedure by Jones et al.[23] via alkylation of *N*-(*tert*-butyloxycarbonyl)hydroxylamine (*N*-Boc-hydroxylamine) with the respective alkyl bromide followed by cleavage of the Boc protecting group.[24] Briefly, 5 g (37.55 mmol) *N*-Boc-hydroxylamine and 15 mmol of the respective alkyl bromide (2.36 mL 1-bromoheptane or 0.76 mL 1-bromo-3-hydroxypropane) were mixed in a round bottom flask. Slowly, 11.2 mL (75.1 mmol) 1,8-diazabicyclo[5.4.0]undec-7-en (DBU) followed by 5 mL dichloromethane were added through a syringe, and the mixture was stirred at room temperature for 24 hours. Afterwards, it was dissolved in 495 mL dichloromethane and this solution was washed twice with 50 mL 1N hydrochloric acid and twice with 75 mL of a saturated solution of sodium chloride. The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated. Boc-HA1: ^{1}H NMR ($CDCl_3$): δ (ppm) = 10.74 (w, br s, NH); 3.77 (t, 2H, C*H*$_2$ONH$_2$, ^3J = 6.6 Hz); 1.55 (tt, 2H, C*H*$_2$CH$_2$ONH$_2$, ^3J = 6.6 Hz); 1.41 (s, 9H, C(C*H*$_3$)$_3$); 1.20 (m, 8H, (C*H*$_2$)$_4$); 0.80 (t, 3H, CH$_2$C*H*$_3$, ^3J = 6.6 Hz). Boc-HA2: ^{1}H NMR ($CDCl_3$): δ (ppm) = 10.72 (w, br s, NH); 7.56 (br s, 1H, OH); 3.99 (t, 2H, C*H*$_2$OH, ^3J = 5.6 Hz); 3.77 (t, 2H, C*H*$_2$ONH$_2$, ^3J = 5.6 Hz); 1.81 (tt, 2H, CH$_2$C*H*$_2$CH$_2$, $^3J_1 = {}^3J_2$ = 5.6 Hz); 1.44 (s, 9H, C(C*H*$_3$)$_3$).

For deprotection of the hydroxylamine, 50 mL 3N hydrochloric acid were added to a solution of the raw product in 50 mL ethyl acetate. This reaction mixture was stirred at room temperature for 1 hour. The organic layer was separated from the aqueous phase and the ethyl acetate was evaporated. The raw product was dissolved in a little amount of methanol and the product was isolated by precipitation into cold ether, yielding 1.39 g (10.6 mmol, 71%) HA1 and 0.51 g (5.6 mmol, 37%) HA2, respectively, of a slightly yellowish solid. HA1: ^{1}H NMR (DMF-d$_7$): δ (ppm) = 11.18 (br s, 2H, N*H*$_2$); 4.25 (t, 2H, C*H*$_2$ONH$_2$, ^3J = 6.7 Hz); 1.63 (tt, 2H, C*H*$_2$CH$_2$ONH$_2$, $^3J_1 = {}^3J_2$ = 6.7 Hz); 1.24 (m, 8H, (C*H*$_2$)$_4$); 0.84 (t, 3H, CH$_2$C*H*$_3$, ^3J = 6.8 Hz). HA2: ^{1}H NMR ($CDCl_3$): δ (ppm)

= 11.09 (br s, 2H, NH_2); 10.40 (br s, 1H, OH); 4.06 (t, 2H, CH_2OH, ^3J = 6.3 Hz); 3.41 (t, 2H, CH_2ONH$_2$, ^3J = 6.3 Hz); 1.69 (tt, 2H, CH$_2$CH_2CH$_2$, $^3J_1 = {}^3J_2$ = 6.3 Hz).

Functionalization of PMSSQ-PDEEA films with hydroxylamines For the functionalization of the deprotected aldehyde groups with hydroxylamines, namely O-heptylhydroxylamine and O-(3-hydroxypropyl)hydroxylamine, the coated glass slides were kept in a 10wt.-% solution of the hydroxylamine in DMF at 50°C overnight. Afterwards, the surface was purged twice with THF and dried with a nitrogen stream.

Functionalization of PMSSQ-PDMA films with amines The deprotected PMSSQ-PDMA films were functionalized with amines similar to the functionalization protocol of PMSSQ-PDEEA films.

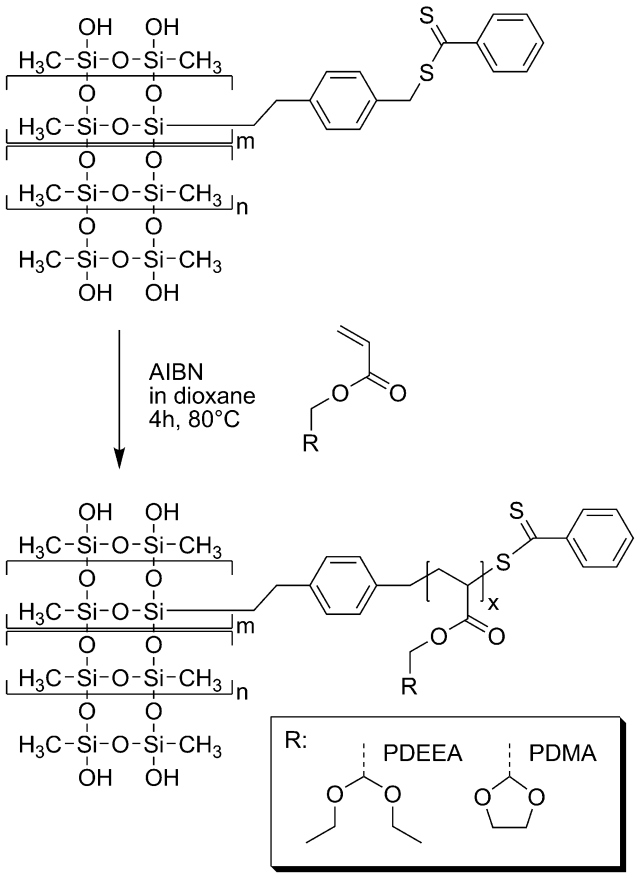
Results and Discussion

Following a recently published synthetic method for the preparation of functional surface coatings using PMSSQ-based hybrid polymers,[8] we plan to enlarge the number of applicable monomers, and thus reactive side groups that can be integrated into these coating materials, by investigation of poly(acrylates) with different acetal side groups. Acetal side groups are of high interest due to their orthogonal reactivity compared to the activated ester monomers, such as pentafluorophenyl acrylate, which has successfully been polymerized from PMSSQ and then been employed as reactive, tunable surface coating.[9,25]

Synthesis of PMSSQ-poly(acrylates) with acetal side groups A PMSSQ macro chain transfer agent (CTA) was synthesized according to a previously published procedure.[8] Briefly, phenyl magnesium bromide was converted with carbon disulfide and the resulting dithiocarboxylate was then reacted with *p*-(chloromethyl)phenylethyl trimethoxy silane to yield the dithiobenzoic acid benzyl-(4-ethyltrimethoxysilyl)ester (RAFT-Si). This chain transfer agent was finally cocondensed with methyl trimethoxy silane (MTMS) under acidic conditions to yield the PMSSQ macro CTA. Noteworthy, this step must not be conducted to complete

conversion. It is important that there are still some free silanol groups which are necessary for the later cross-linking and thus stabilization of the hybrid films by thermal curing after spin-coating.

The PMSSQ macro CTA enabled the RAFT polymerization of the acrylate monomers from defined sites in the functionalized PMSSQ network. Namely, 2,2-diethoxyethyl acrylate (DEEA) and 1,3-dioxolan-2-ylmethyl acrylate (DMA) were polymerized using the PMSSQ macro CTA, yielding hybrid polymers with diethyl acetal and cyclic ethylene acetal side groups, respectively (see **Scheme 1**). The copolymers were characterized by ^{1}H NMR and GPC (see supporting information, **Figure S1-4**).



SCHEME 1: RAFT polymerization of the vinyl monomers from a PMSSQ macro chain transfer agent.

Film preparation The PMSSQ-copolymers were dissolved in THF at a concentration of 10wt.-% and spin-coated onto previously cleaned glass substrates. To stabilize the coatings via cross-linking of the remaining silanol groups, the films were cured

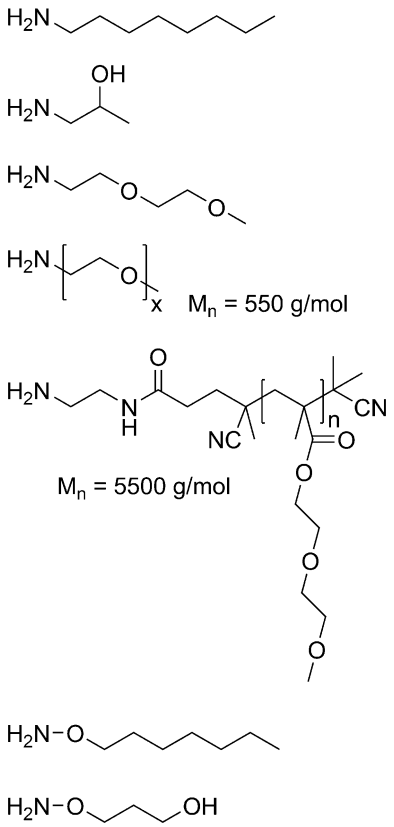
thermally at 130°C. The adherence to the glass surface was tested via the standardized ISO tape test.[26] All polymer types could easily be detached from the surface prior to curing, but showed no tearing at all after thermal cross-linking, and therewith can be considered as stable surface coatings. Moreover, the surface roughness of the cured films (on silicon wafers) was determined by AFM and spin-coating both hybrid polymers resulted in smooth films. The root-mean-square deviation of the surface roughness (RMS) on a 10 x 10 μm^2 square of the PMSSQ-PDEEA film was 0.4 nm and 0.6 nm of the PMSSQ-PDMA film (see supporting information, **Figures S5**, **S6**).

Deprotection and functionalization of PMSSQ-PDEEA films with amines The diethyl acetals in the PMSSQ-PDEEA films were deprotected via solution dipping in diluted trifluoroacetic acid for one hour, followed by intensive washing with deionized water to remove excess TFA. The highest efficiency for the deprotection of diethyl acetals was found for a ratio of TFA to water of 2:1 and 3:1 (see **Table 1**).

TABLE 1: Comparison of deprotection procedures (each 1 hour) for the two types of acetals.

Protecting group	TFA : H_2O	Deprotection
diethyl acetal	1 : 3	No
diethyl acetal	1 : 1	Partial
diethyl acetal	2 : 1	Yes
diethyl acetal	3 : 1	Yes
ethylene acetal	1 : 3	No
ethylene acetal	1 : 1	No
ethylene acetal	2 : 1	No
ethylene acetal	3 : 1	Partial
ethylene acetal	6 : 1	Partial
ethylene acetal	8 : 1	Yes
ethylene acetal	9 : 1	Yes

Afterwards, the aldehyde groups could be functionalized with a variety of primary amines (see **Scheme 2**). For this, the substrate was placed in a 10wt.-% THF solution of the respective amine at room temperature for one hour (exception: NH_2-PEG of MW ~ 550 g/mol was allowed to react with the surface for two hours).



SCHEME 2: Reagents (primary amines, amine-terminated polymers and hydroxylamines) used for the functionalization of the aldehyde groups.

As an example, the conversion with octylamine will be discussed in detail. The reaction steps were monitored via IR spectroscopy (**Figure 1**). All spectra showed very broad bands around 905, 760, and 410 cm^{-1}, representing the different Si-O-vibrations of the inorganic block, in the fingerprint region. In addition, the spectrum of the original PMSSQ-PDEEA film (**Figure 1a**) exhibits a carbonyl band at 1738 cm^{-1}, corresponding to the ester groups of the poly(acrylate) block, and a pattern of three bands characteristic of a diethyl acetal between 2820 and 3000 cm^{-1}. Even though, the intensity of the latter bands was low, due to the ATR setup, they disappeared

completely during the deprotection of the acetal side groups (**Figure 1b**). After the surface functionalization with octylamine, the IR spectrum of the film showed strong bands at 2920 and 2850 cm^{-1}, which correspond to the CH_2-stretching of the octyl chain (**Figure 1c**).

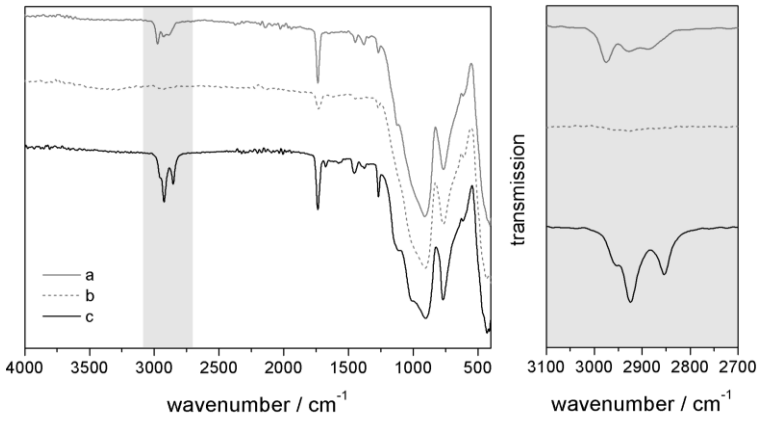


FIGURE 1: IR spectra of the PMSSQ-PDEEA films a) before, b) after acidic deprotection and, c) after deprotection and functionalization with octylamine.

The AFM image of the functionalized surface showed a slight increase in roughness (RMS = 3.2 nm, see supporting information, **Figure S7**) compared to the freshly cured film, however, the RMS value was still lower than those of regular glass (~5 nm) or polymeric substrates (>10 nm) and the functionalized film was still considered as being smooth.

The surface properties can be varied flexibly depending on the respective amine used. As an example, the surface hydrophilicity was tuned by the reaction of the coating material with amines carrying hydrophilic and hydrophobic moieties, respectively. As a measure of the surface hydrophilicity, the advancing contact angle Θ_a (see **Figure 2**) was investigated. On a bare PMSSQ-PDEEA surface, the advancing contact angle Θ_a was found to be 106° and to decrease to 90° after the deprotection of the acetal groups. When the resulting aldehyde groups were converted with hydrophilic amines, such as 2-hydroxypropylamine, di(ethylene glycol) methyl ether amine, and a PEG amine (MW ~ 550 g/mol), the obtained surfaces showed advancing contact angles Θ_a of 75°, 69°, and 42°, respectively. And after the reaction with

octylamine, the surface was found to be hydrophobic with an advancing contact angle Θ_a of 113°.

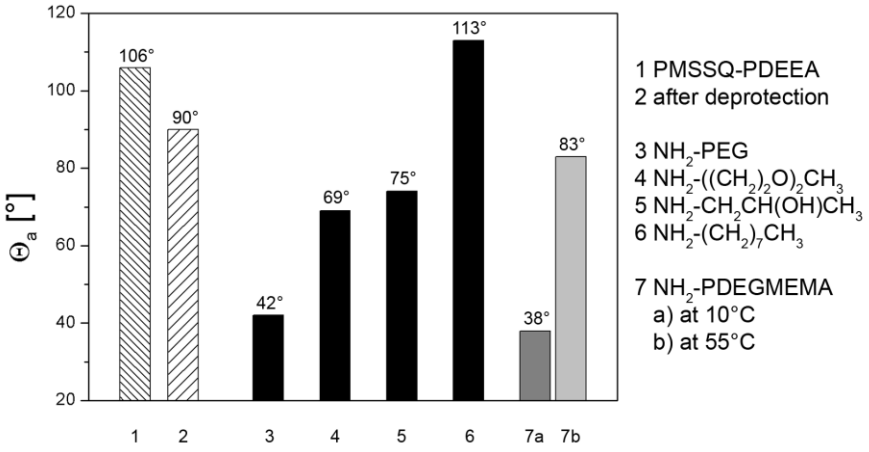


FIGURE 2: Advancing contact angles of PMSSQ-PDEEA films before and after acidic deprotection and after surface functionalization with different amines (after acidic deprotection).

A stimuli-responsive surface with a switchable hydrophilicity was obtained by reaction of a deprotected PMSSQ-PDEEA film with the thermo-responsive polymer PDEGMEMA, which was functionalized with an amino group at one chain end. PDEGMEMA exhibits a lower critical solution temperature (LCST) at 26°C, which means it is soluble in water below the LCST and precipitates in water above the LCST. Since the amino group at the chain end of the polymer (M_n = 5500 g/mol) is not as freely available as the one in small molecular amines, due to the conformation of a random coil in solution, the substrate was allowed to react with the polymer solution for two hours instead of only one hour. Afterwards, the advancing contact angle Θ_a was measured at T = 10°C (T < LCST) and T = 55°C (T > LCST), respectively, by cooling or heating the coated glass slide with a Peltier element during the contact angle measurements. As expected, the surface exhibited a hydrophilic behavior (Θ_a = 38°) at temperatures below the LCST of the stimuli-responsive polymer and turned into a more hydrophobic surface (Θ_a = 83°) above the LCST. The change of the advancing contact angle was fully reversible and could be switched back and forth by repetitive heating and cooling, as confirmed by five cycles.

Functionalization of PMSSQ-PDEEA films with hydroxylamines Further, hydroxylamines were used for the conversion of the aldehyde groups to result in the respective oximes. For the surface functionalization with hydroxylamines, harder conditions were required in comparison to the reactions with amines. For good conversion, the substrate exhibiting the aldehyde groups, that were deprotected as described above, had to be heated in a 10wt.-% DMF solution of the respective hydroxylamine to 50°C overnight. If the reaction was conducted at room temperature, only a very low conversion could be achieved after 2 hours and even after one night. As an example, the reaction of a previously deprotected PMSSQ-PDEEA film with *O*-heptylhydroxylamine was followed by IR spectroscopy (**Figure 3**) and the conversion was indicated by the intensity of the CH_2-stretching bands between 2790 and 3000 cm^{-1} representing the alkyl chain of *O*-heptylhydroxylamine and the appearance of a new band at 1612 cm^{-1}, which can be assigned to the oxime bond (C=N).

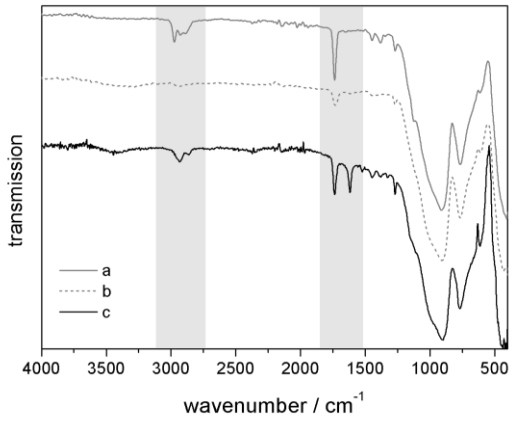


FIGURE 3: IR spectra of the PMSSQ-PDEEA films a) before, b) after acidic deprotection and, c) after deprotection and functionalization with O-heptylhydroxylamine.

Despite clear indication of the conversion with the hydroxylamine in the IR spectrum, the advancing contact angle Θ_a was found to be only 91° and thus much lower than the contact angle, which had been achieved during the functionalization with octylamine. After the conversion with a more hydrophilic hydroxylamine,

O-(3-hydroxypropyl)hydroxylamine, the film surface exhibited a contact angle Θ_a of 81°, which is higher than the one expected from the comparison with the reaction with 2-hydroxypropylamine. Thus, according to our observations, the reaction with hydroxylamines was not as capable to tune the surface hydrophilicity of our coatings as the conversion with amines. Additionally, it should be mentioned in this context that a large number of different amines is commercially available, while most hydroxylamines need to be synthesized, for example from the corresponding bromides.

Deprotection and functionalization of PMSSQ-PDMA films Recently, synthetic strategies towards multifunctional or "smart" surface coatings gained more and more interest due to their high potential for high-end applications like microfluidic devices ("lab-on-a-chip" systems) or biomedical devices for diagnosis.[1,2,27,28] In order to obtain such multifunctional surfaces, selectively addressable functional groups in the coating material are required. As shown by Kametani et al.,[17] cyclic ethylene acetals are more stable than dialkyl acetals under hydrolytic conditions and, as an example, dimethyl and diethyl acetals could be cleaved selectively in the presence of a 1,3-dioxolane group. Thus, we assumed that the cyclic ethylene acetal group in the PMSSQ-PDMA films should be more stable than the diethyl acetal group in PMSSQ-PDEEA films under acidic conditions and therewith these two polymers should be good candidates for the preparation of multifunctional surfaces via selectively addressable functional groups.

For the deprotection of the cyclic acetals in PMSSQ-PDMA films, several experiments with different ratios of acid (TFA) to water were tested (**Table 1**) and among these experiments, the highest efficiency for the deprotection of the PMSSQ-PDEEA films was determined. In all the tests, the coated substrates were placed into the respective deprotection mixture for one hour at room temperature and afterwards washed intensively with deionized water to remove excess of TFA. The efficiency of the deprotection reactions was deduced from IR spectroscopy. As expected, the cyclic ethylene acetal side groups showed higher stability than the diethyl acetals and

could not be deprotected with mixtures with low TFA content (TFA : H_2O = 1:3, 1:1, 2:1). At a percentage of 75% TFA content, the cyclic ethylene acetal was cleaved partially and with mixtures with a higher TFA content (TFA : H_2O = 8:1 or 9:1), the ethylene acetals could be fully deprotected and subsequently functionalized with high efficiency. Summarizing the results of the deprotection tests on the two different polymers, PMSSQ-PDEEA and PMSSQ-PDMA, a mixture of TFA with water in the ratio of 2:1 is an appropriate reagent for the deprotection of the diethyl acetals and does not cleave the cyclic acetals, while a mixture of TFA with water in the ratio of 8:1 was sufficient to deprotect both acetals yielding the aldehyde group.

The surface resulting from the deprotection of PMSSQ-PDMA was chemically identical to the one resulting from the deprotection of PMSSQ-PDEEA and thus the methods discussed for the functionalization of the aldehyde groups with amines and hydroxylamines were equally applicable on the deprotected PMSSQ-PDMA films and resulted in surfaces with comparable properties as determined by contact angle measurements.

Herewith, we found a system of two different protecting groups for aldehyde side groups that can be deprotected selectively and thus allow independent functionalization with different nucleophiles as shown in the functionalization of coating materials. More precisely, on a surface exhibiting diethyl acetal and cyclic ethylene acetal groups, this synthetic toolbox would enable us to cleave the more labile diethyl acetals first using a weaker TFA solution (2:1) and then to functionalize the resulting aldehyde groups without deprotection of the cyclic acetals, which could be deprotected and functionalized afterwards using a higher concentrated TFA solution (8:1).

Conclusion

Two inorganic/organic copolymers could be synthesized via RAFT polymerization from 2,2-diethoxyethyl acrylate and 1,3-dioxolan-2-ylmethyl acrylate, respectively, using a PMSSQ-based macro chain transfer agent. These hybrid polymers could be

employed for the preparation of smooth surface coatings which were successfully stabilized thermally. The films containing different types of acetal groups could be functionalized easily after acidic deprotection via conversion of the resulting aldehyde groups with a variety of amines and hydroxylamines. Both reaction steps were performed by simple solution-dipping. This method could be applied to tune the surface hydrophilicity depending on the respective reagent used for the functionalization reaction and to prepare a thermo-responsive surface based on PDEGMEMA. Furthermore, the two types of acetal side groups investigated in these studies could be addressed independently. Based on their different stability towards acid treatment, deprotection procedures applicable on the two types of acetals could be developed which allow the exclusive deprotection of the diethyl acetal and its functionalization without deprotection of the cyclic ethylene acetal. Hence, the system of the two hybrid copolymers discussed provides a synthetic platform for the preparation of multifunctional surface coatings.

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[5] In the meantime, published as: D. Kessler, P. Theato, *Langmuir* **2009**, *25*, 14200-14206.

Supporting Information

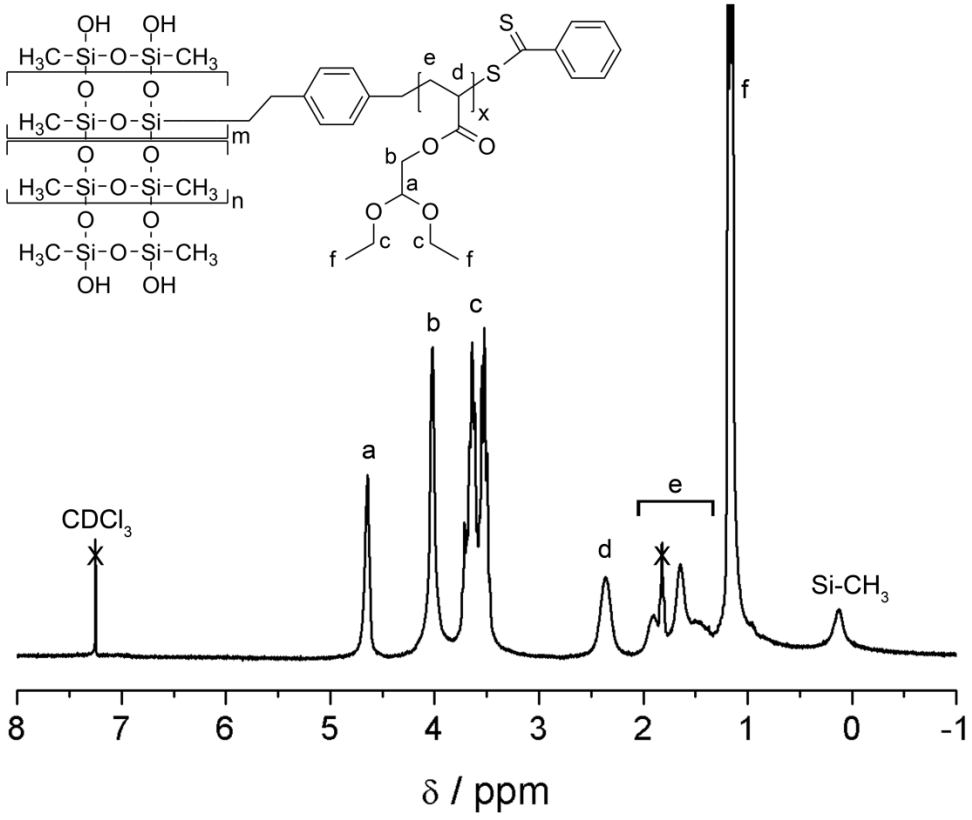

FIGURE SI: ^{1}H NMR spectrum of the PMSSQ-PDEEA copolymer.

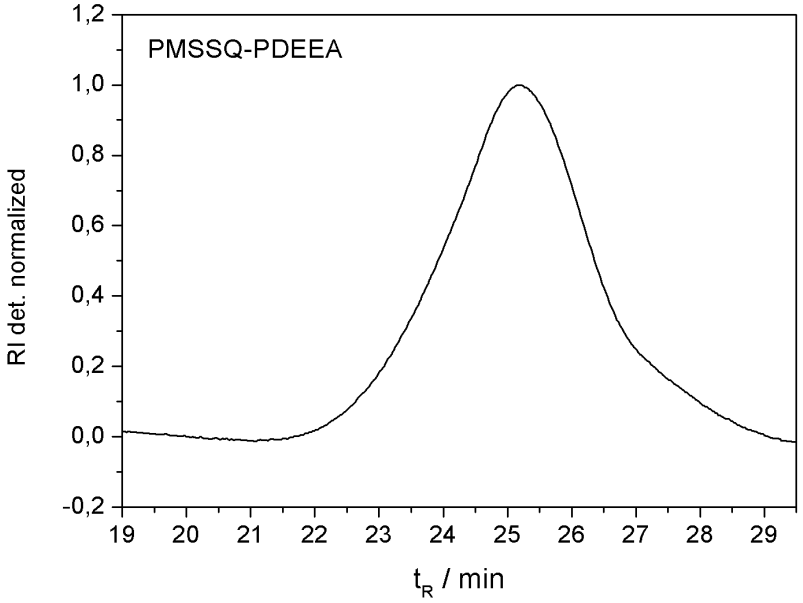

FIGURE S2: GPC elugram of the PMSSQ-PDEEA copolymer (M_n = 30100, M_w = 42000, PDI = 1.39).

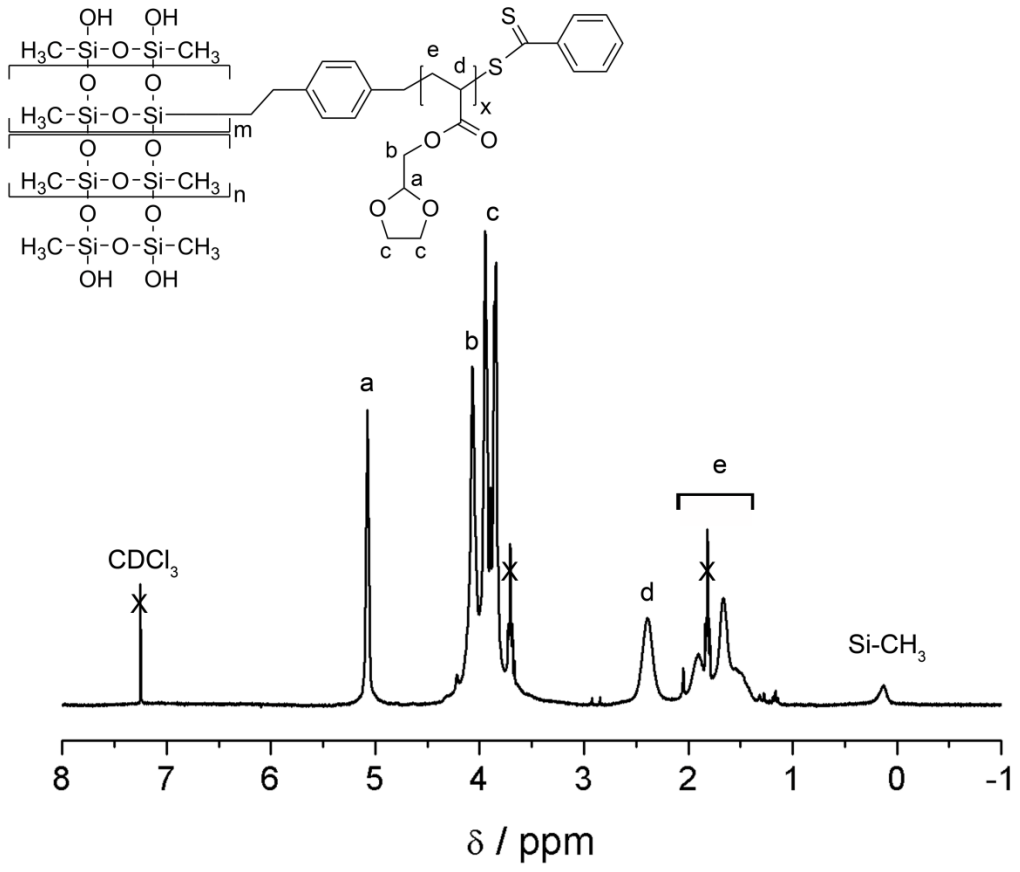

FIGURE S3: ^{1}H NMR spectrum of the PMSSQ-PDMA copolymer.

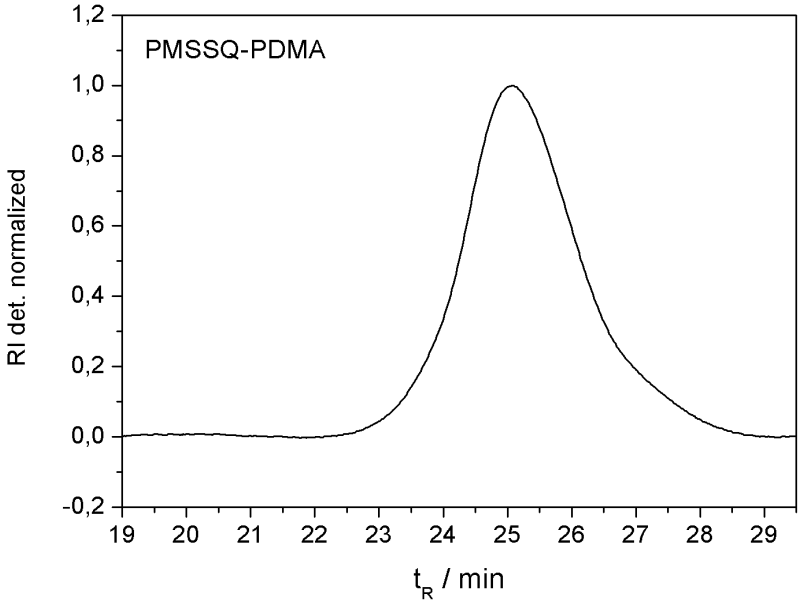

FIGURE S4: GPC elugram of the PMSSQ-PDMA copolymer (M_n = 37100, M_w = 59600, PDI = 1.61).

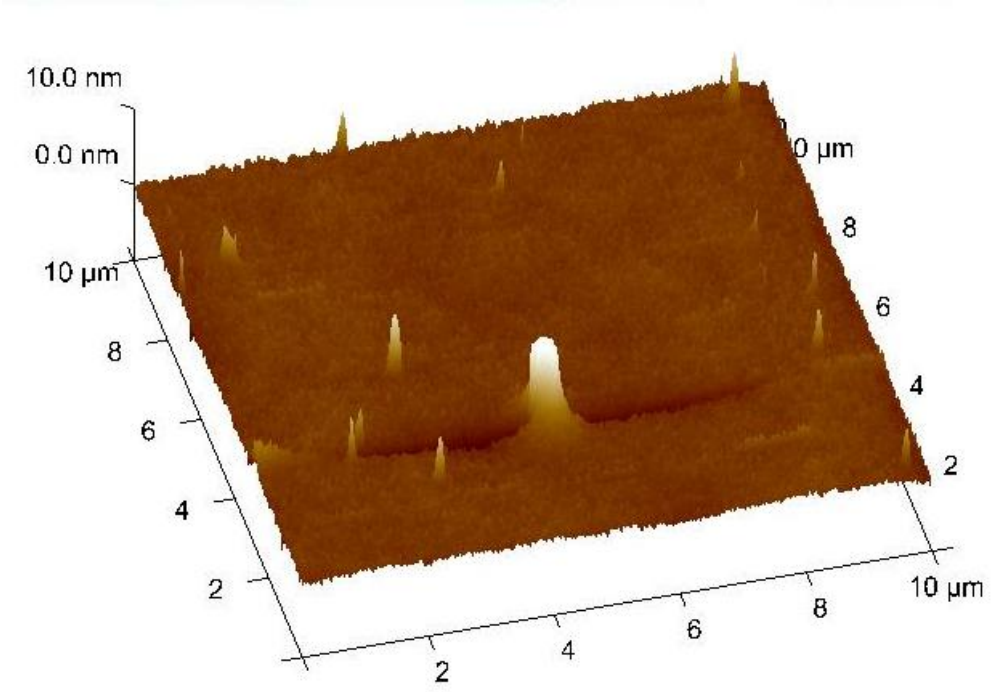

FIGURE S5: AFM topography of the PMSSQ-PDEEA copolymer on silicon after curing.

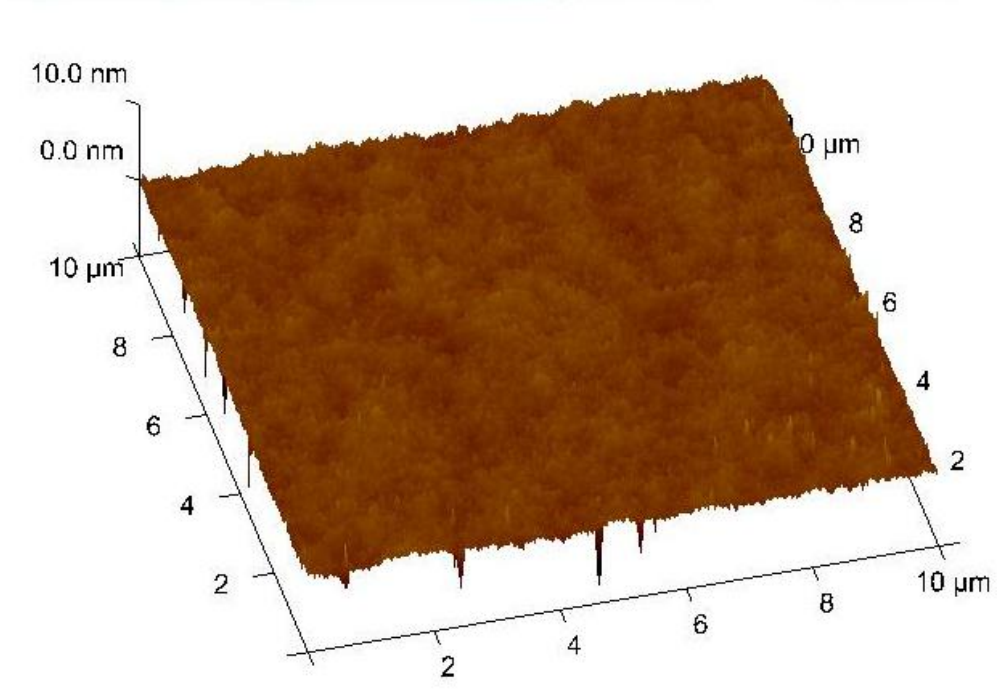

FIGURE S6: AFM topography of the PMSSQ-PDMA copolymer on silicon after curing.

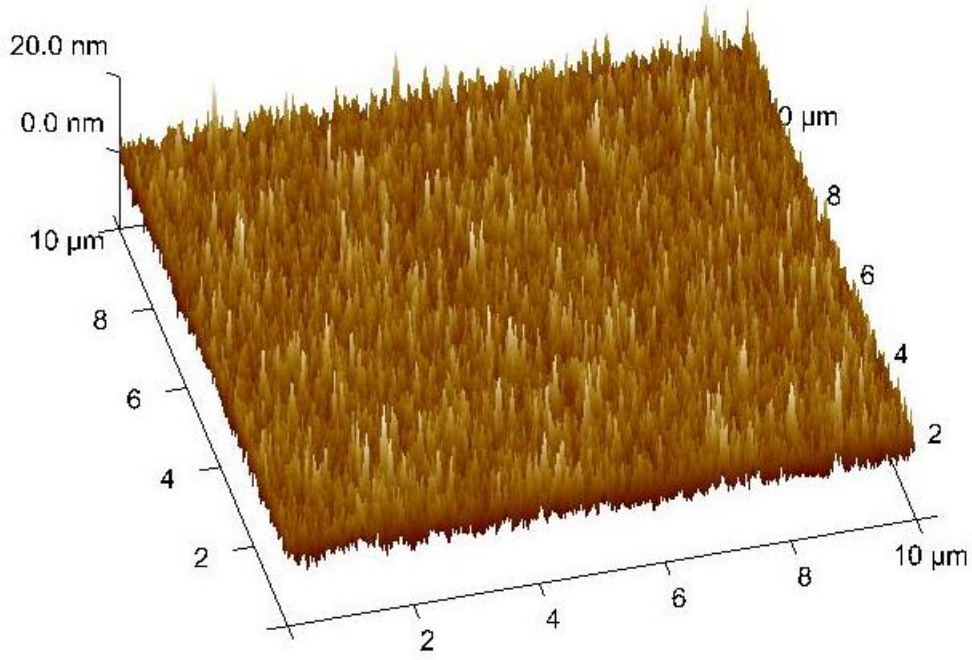

FIGURE S7: AFM topography of the PMSSQ-PDEEA copolymer on silicon after curing and conversion with octylamine.

5 Summary and Conclusion

Efficient methods for the functionalization of both ends of a polymer chain that are suitable for polymer and bioconjugation were developed for polymers prepared via reversible addition-fragmentation chain transfer (RAFT) polymerization. For this purpose, a dithioester-based chain transfer agent (CTA) with an activated ester moiety in the R-group, namely pentafluorophenyl-(4-phenylthiocarbonylthio-4-cyanovalerate) (PFP-CTA), was synthesized and its application as universal tool for the functionalization of the α-end-group was demonstrated. On the one hand, it was shown, how this PFP-CTA can be used as precursor for the design of other functional CTAs via straightforward aminolysis of the activated ester, and thus reduces the synthetic effort usually associated with the development of a new CTA. The conversion into an alkyne-CTA, which itself is a useful tool for controlled radical polymerization and modification of the α-chain end as well as polymer conjugation via 1,3-dipolare cyclodaddition, could be achieved with nearly quantitative yield in a one-step reaction. On the other hand, the PFP-CTA was employed successfully in the controlled polymerization of several methacrylate monomers via the RAFT process yielding polymers with a narrow molecular weight distribution and well-defined reactive α-end-groups. This chain end could then be converted with different primary amines such as propargyl amine, 1-azido-3-aminopropane or ethylene diamine, resulting in polymers with alkyne-, azide- or amine-functionalized α-chain end, respectively, or directly with the amine end-groups of different peptides, resulting in polymer-peptide-conjugates. These reactions could be monitored via ^{1}H

and ^{13}C NMR, and IR spectroscopy as well as via ^{19}F NMR spectroscopy, which indicated quantitative consumption of the pentafluorophenyl ester group. To avoid undesired side reactions of the dithioester ω-end-group during these reactions at the α-chain end, the dithioester could be radically substituted via simple treatment with an excess of 2,2'-azobisisobutyronitrile (AIBN) prior to aminolysis of the α-chain end. The described alkyne-CTA was also employed for the RAFT polymerization of different methacrylate monomers, and the successful incorporation of the alkyne functionality at the α-end-group as well as its stability toward the reaction conditions of the excessive AIBN treatment could be confirmed via ^{1}H and ^{13}C NMR spectroscopy as well as MALDI TOF mass spectroscopy. In these cases, the radical substitution of the dithioester end-group was performed to avoid potential interaction with the catalyst of the subsequent copper-catalyzed azide alkyne cycloaddition (CuAAC) reactions, for which the alkyne-end-group was introduced. As a proof of principle, the conjugation of such an alkyne-terminated poly(diethylene glycol methyl ether methacrylate) (PDEGMEMA) with an azide-terminated poly(*tert*-butyl methacrylate), which was obtained via conversion of an activated ester end-group with 1-azido-3-aminopropane, was conducted via CuAAC. Isolation of the resulting diblock copolymer via precipitation allowed for complete separation from building block 1, which was used in excess. As a consequence of the experimental design, only a very small amount (< 2wt.-%) of unreacted building block 2 was left. Besides a successful polymer conjugation reaction, this again demonstrates the efficiency of the end-group functionalization using the activated ester α-end-group.

Furthermore, via direct conversion of pentafluorophenyl ester-functionalized stimuli-responsive polymers, namely PDEGMEMA and the more hydrophilic poly(oligoethylene glycol methyl ether methacrylate) (POEGMEMA), with collagen-like peptides, well-defined polymer-peptide diblock copolymers as well as polymer-peptide-polymer triblock copolymers could be prepared. Appropriate reaction conditions and elegant isolation procedures were found, so that the synthesis of diblock copolymers was quantitative in terms of end-group conjugation, the

synthesis of triblock copolymers yielded more than 86% of the desired triblock structures with only an impurity of less than 14% diblock copolymer, and all the products could be separated completely from unreacted homopolymer, which was used in excess. Subsequently, the side groups in the peptide segments could be deprotected under acidic conditions. The stability of the polymer blocks under these conditions was verified.

As known from natural collagen and the pure collagen-like peptide employed here, the PDEGMEMA-based hybrid copolymers formed trimers containing collagen-like triple-helices in cold aqueous solution, which was confirmed via circular dichroism (CD) spectroscopy. Temperature-dependent CD spectroscopy, turbidimetry, and dynamic light scattering indicated that both bioconjugates self-assembled into double stimuli-responsive superstructures at higher temperatures, which underwent at least two conformational transitions upon heating. At temperatures above 30°C, superstructures in the size range of several hundred nanometers were observed. Their formation was most likely driven by the temperature induced collapse of the polymer blocks. In the case of the PDEGMEMA-*b*-collagen-*b*-PDEGMEMA triblock copolymer, the hypothesis, that these superstructures were large spherical or worm-like micelles, was supported by first TEM studies. Upon heating to temperatures above 50°C and thus above the melting temperature of the collagen-like helices, smaller superstructures were found in the solutions of both bioconjugates, and after cooling back down to 10°C, the original trimeric state was recovered.

As expansion of the synthetic strategy for bioconjugation via the combination of activated ester chemistry and RAFT polymerization, homotelechelic PDEGMEMA with two pentafluorophenyl ester end-groups was prepared. This was achieved by using the PFP-CTA for the functionalization of the α-end-group and radical substitution of the dithioester ω-end-group via excessive treatment with a functional derivative of AIBN. The conversion of both reactive chain ends with an N-terminus of a peptide segment resulted in a peptide-polymer-peptide triblock copolymer.

Finally, also the straightforward preparation of peptide-reactive surfaces on the basis of inorganic-organic hybrid materials, namely PMSSQ-poly(2,2-diethoxyethyl acrylate) (PMSSQ-PDEEA) and PMSSQ-poly(1,3-dioxolan-2-ylmethyl acrylate) (PMSSQ-PDMA), could be demonstrated. Spin-coating of 10 wt.-% solutions of these copolymers and thermally induced crosslinking resulted in smooth, robust surface coatings as tested via atomic force microscopy and a standardized ISO tape test, respectively. After acidic deprotection of the acetal moieties in these films, the resulting aldehyde groups could be converted with a variety of amines and hydroxylamines via simple solution dipping, which was used for modification of the surface hydrophilicity depending on the respective reagent used and for preparation of a stimuli-responsive surface based on PDEGMEMA. Moreover, based on the different stability of the two types of acetal groups compared in these studies, deprotection procedures could be developed, which enable the exclusive deprotection of the diethyl acetals in PMSSQ-PDEEA and their conversion without deprotection of the cyclic ethylene acetals in PMSSQ-PDMA. Hence, the combination of these two hybrid copolymers provides a synthetic platform for the preparation of multifunctional surface coatings, which are promising candidates for applications involving protein immobilization due to the discussed peptide-reactive handles in these robust films.

In summary, via combination of the RAFT process with reactive moieties such as the activated pentafluorophenyl ester and two types of acetal-protected aldehydes, a versatile toolbox for precise modification of polymer end-groups, polymer and bioconjugation and the immobilization of proteins on functional surfaces could be expanded with the particular interest in bioconjugates based on a collagen-like peptide and their double stimuli-responsive self-assembly behavior. This approach also facilitates the design of further functional CTAs and allows for a high combinatory flexibility, which is especially valuable for the exploration of further coupling strategies for polymer and bioconjugation.

6 Publications

1. Kerstin Wiss, Daniel Kessler, Patrick Theato "Synthesis of Hybrid Polymers Containing Acetal Side Groups for the Design of Versatile Responsive Surfaces" *PMSE Preprints* **2008**, *99*, 74-75.
2. Peter J. Roth, Kerstin T. Wiss, Rudolf Zentel, Patrick Theato "Synthesis of Reactive Telechelic Polymers based on Pentafluorophenyl Esters" *Macromolecules* **2008**, *41*, 8513-8519.
3. Kerstin T. Wiss, Ohm D. Krishna, Peter J. Roth, Kristi L. Kiick, Patrick Theato "A Versatile Grafting-to Approach for the Bioconjugation of Polymers to Collagen-like Peptides Using an Activated Ester Chain Transfer Agent" *Macromolecules* **2009**, *42*, 3860-3863.
4. Kerstin T. Wiss, Daniel Kessler, Timothy J. Wendorff, Patrick Theato "Versatile Responsive Surfaces via Hybrid Polymers Containing Acetal Side Groups" *Macromol. Chem. Phys.* **2009**, *210*, 1201-1209.
5. Ohm D. Krishna, Kerstin T. Wiss, Peter J. Roth, Patrick Theato, Kristi L. Kiick "Assembly of Thermally Responsive, Collagen Peptide-containing Block Copolymers" *PMSE Preprints* **2009**, *101*, 1477-1478.
6. Ohm D. Krishna, Kerstin T. Wiss, Peter J. Roth, Patrick Theato, Kristi L. Kiick "Conformational and Assembly Behavior of Collagen-mimetic Peptides and their Thermally Responsive Polymer Conjugates" *Polymer Preprints* **2010**, *51*, 49-50.

7. Kerstin T. Wiss, Lisa zur Borg, Peter J. Roth, Patrick Theato "Combining RAFT Polymerization and Activated Ester Chemistry to a Versatile Tool for Polymer Conjugation" *Polymer Preprints* **2010**, *51*, 318-319.
8. Kerstin T. Wiss, Patrick Theato "Facilitating Polymer Conjugation via Combination of RAFT Polymerization and Activated Ester Chemistry" *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *accepted* (DOI 10.1002/pola.24267).
9. Peter J. Roth, Kerstin T. Wiss, Patrick Theato "Polymer Analogous Reactions" contribution in *Comprehensive Polymer Science*, Elsevier **2010** *submitted.*

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- [redacted] and [redacted] for an intensive cooperation and an inspiring research stay at the University of Delaware, Newark,

- [redacted] (Humboldt University, Berlin / Max Planck Insitute for Colloids and Interfaces, Postdam) for good collaboration,
- [redacted] and the members of the groups of [redacted] and especially [redacted] for scientific discussion and exchange of ideas,
- [redacted] for their support during their internships,
- [redacted] for technical support.

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