# Bioapplications of RAFT Polymerization

Cyrille Boyer,[†] Volga Bulmus,[‡] Thomas P. Davis,*[,†] Vincent Ladmiral,[§] Jingquan Liu,[†] and Sébastien Perrier*[,§]

*Centre for Advanced Macromolecular Design (CAMD), School of Chemical Sciences & Engineering, UNSW, Sydney, NSW 2052, Australia, Centre for Advanced Macromolecular Design (CAMD), School of Biotechnology & Biomolecular Sciences, UNSW, Sydney, NSW 2052, Australia, and Key Centre for Polymers & Colloids, School of Chemistry, Building F11, Eastern Avenue, The University of Sydney, NSW 2006, Australia*

*Received April 6, 2009*

## Contents

1. Introduction 5402
1.1. Reversible Addition−Fragmentation Chain Transfer (RAFT) Polymerization 5402
1.1.1. Mechanism of RAFT 5404
1.1.2. Choice of RAFT Agents 5404
1.1.3. Monomers 5405
1.1.4. Polymer Functionality and Architecture 5406
1.2. Application of the RAFT Technique to Biological Applications 5407
1.3. Scope of the Review 5407
2. Functional Polymers Obtained by RAFT Polymerization 5407
2.1. End-Group Functionalization 5407
2.1.1. Functional RAFT Agents 5408
2.1.2. RAFT Design Allowing a Direct Conjugation 5409
2.1.3. Synthesis of Biohybrid RAFT Agents 5410
2.1.4. Chemical Modification of a RAFT End Group 5411
2.2. Pendant Groups Functionalization 5413
2.2.1. Thiol Reactive Monomers 5414
2.2.2. Activated Ester Monomers 5414
2.2.3. Amine Functionalized Monomers 5415
2.2.4. “Clickable” Monomers 5415
2.2.5. “Unusual” Monomers 5415
3. Bioconjugates 5416
3.1. Fabrication of Protein−Polymer Conjugates 5417
3.1.1. Postpolymerization Conjugation Methodology 5417
3.1.2. In Situ Polymerization Methodology 5419
3.2. Biotinylated Polymers for Conjugation to (Strept)avidin 5419
3.3. Peptide−Polymer Conjugates 5420
3.4. Folate Functionalized Assemblies 5420
3.5. DNA/RNA Conjugates 5420
3.6. Glycopolymers 5421
4. Drug Delivery 5423
4.1. Supramolecular Assemblies: Micelles, Vesicles, and Stars 5424
4.1.1. Stealth Micelles/Vesicles 5424
4.1.2. Stimuli-Responsive Micelles/Vesicles/Stars 5425
4.1.3. Corona and Surface Functionalized Micelles/Vesicles 5427
4.2. Particles/Capsules/Gels 5427
4.3. Soluble Polymer−Drug Conjugates 5428
5. Surface Modification by RAFT Polymerization for Biological Applications 5428
5.1. Flat Surfaces 5428
5.2. Particle Surfaces 5429
6. Conclusion and Outlook 5431
7. Abbreviations 5431
8. References 5431

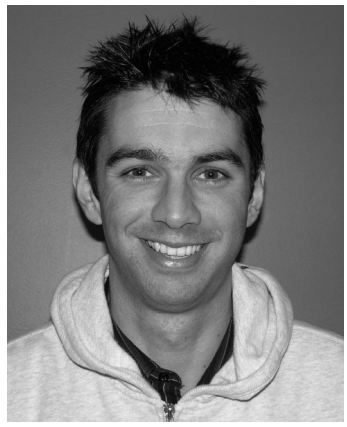
* E-mail: T.P.D., T.Davis@UNSW.edu.au; S.P., S.Perrier@chem.usyd.edu.au.
[†] Centre for Advanced Macromolecular Design (CAMD), School of Chemical Sciences & Engineering, UNSW.
[‡] Centre for Advanced Macromolecular Design (CAMD), School of Biotechnology & Biomolecular Sciences, UNSW.
[§] The University of Sydney.

## 1. Introduction

### 1.1. Reversible Addition−Fragmentation Chain Transfer (RAFT) Polymerization

A living radical polymerization (LRP) is a free radical polymerization that aims at displaying living character, (i.e., does not terminate or transfer and is able to continue polymerization once the initial feed is exhausted by addition of more monomer). However, termination reactions are inherent to a radical process, and modern LRP techniques seek to minimize such reactions, therefore providing control over the molecular weight and the molecular weight distribution of a polymeric material. In addition, the better LRP techniques incorporate many of the desirable features of traditional free radical polymerization, such as compatibility with a wide range of monomers, tolerance of many functionalities, and facile reaction conditions. The control of molecular weight and molecular weight distribution has enabled access to complex architectures and site specific functionality that were previously impossible to achieve via traditional free radical polymerizations. These LRPs are classified in three different subgroups: (1) stable free-radical polymerization such as nitroxide mediated polymerization (NMP),[1,2] (2) degenerative transfer polymerization, such as iodine transfer polymerization (ITP and RITP),[3,4] single electron transfer−degenerative transfer living radical polymerization (SET-DTLRP),[5,6] reversible addition−fragmentation chain transfer (RAFT),[7,8] and macromolecular design via the interchange of xanthates (MADIX)[9,10] polymerization, and (3) metal mediated catalyzed polymerization, such as atom transfer radical polymerization (ATRP),[11−14] single electron transfer−living radical polymerization (SET-LRP),[15] and organotellurium mediated living radical polymrization[16−19] Among the existing LRP techniques, RAFT and MADIX are probably the most versatile processes, as they are tolerant

10.1021/cr9001403 CCC: $71.50 
Published on Web 09/18/2009



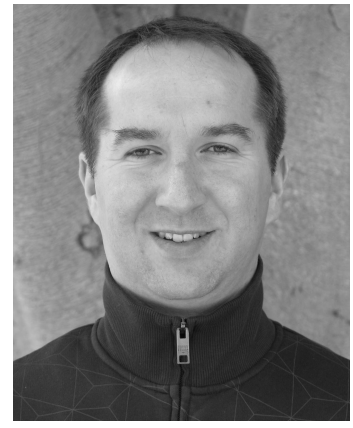
Cyrille Boyer received his Ph.D. in polymer chemistry in 2005 from the University of Montpellier II (Ecole National Supèrieure de Chimie de Montpellier). His Ph.D. was in collaboration with Solvay-Solexis and devoted to the synthesis of new graft copolymers using grafting "to". In 2005, he undertook a postdoctorate position with Dupont Performance and Elastomers (Willmington, United States) and Dr. B. Ameduri dealing with the synthesis of original fluorinated elastomers using controlled radical polymerization (e.g., iodine transfer polymerization). Since October 2006, he has been a senior research fellow under the direction of Prof. Thomas Davis in the Centre of Advanced Macromolecular Design (CAMD), University of New South Wales. His research interests mainly cover the preparation of well-defined polymers, protein−polymer conjugates, and hybrid organic−inorganic nanoparticles using controlled radical polymerization. He has coauthored over 40 peer-reviewed research papers, including 2 book chapters, and 2 patents.

Volga Bulmus received her B.E. and M.Sc. in Chemical Engineering and her Ph.D. in bioengineering (Hacettepe University, Turkey), in 2000. She worked as a postdoctoral research fellow in the Bioengineering Department at the University of Washington between 2001 and 2003. In 2004, she was granted a highly competitive The University of New South Wales (UNSW) Vice Chancellor's Research Fellowship (Australia). In 2008, she was appointed as a Senior Lecturer at the School of Biotechnology and Biomolecular Sciences (UNSW). She is also an adjunct member of The Centre for Advanced Macromolecular Design (CAMD) at UNSW. Dr. Bulmus leads a group of 5−10 researchers working on the development of advanced polymers for biotechnology and biomedical applications. She has published over 45 peer reviewed research papers. Her research interests include design, synthesis, and evaluation of well-defined polymeric systems for nanobiotechnology and drug delivery applications ranging from antitumor chemotherapy and gene silencing to bioseparations and biosensors.

of a wide variety of reaction conditions and functionalities, which enables control over the largest variety of monomers of all the LRP techniques.

RAFT[7,8] and MADIX[9,10] polymerizations were first reported in 1998 by the CSIRO group and Rhodia Chimie, respectively. Both systems proceed via degenerative transfer

Tom Davis has been an academic at UNSW for 17 years following a stint in industry as a research manager at ICI in the U.K. He has coauthored 315+ reviewed papers, patents, and book chapters. He is the Director of the Centre for Advanced Macromolecular Design (CAMD) at UNSW—a Centre with expertise in bio/organic polymer synthesis and polymerization kinetics. He is also a visiting Professor at the Institute for Materials Research & Engineering (IMRE) in Singapore. In 2005 he was awarded a Federation Fellowship by the Australian Research Council. He serves (or has served) on the editorial advisory boards of *Macromolecules*, *Journal of Polymer Science*, *Australian Journal of Chemistry*, *Journal of Materials Chemistry*, and *Journal of Macromolecular Science—Reviews*.



Vincent Ladmiral obtained his diplome d'ingénieur in 1998 from the Chemistry School of Montpellier (France), where he specialized in catalysis. In 2006, he completed his Ph.D. under the supervision of Professor D. M. Haddleton at the University of Warwick, U.K., where he studied and developed living radical polymerizations in combination with click chemistry. He then joined Professor Fukuda's laboratory, Kyoto University, Japan, and applied surface-initiated polymerization to the synthesis of hybrid nanoparticles. In October 2007, he started a postdoctoral fellowship at the University of Leeds, U.K., to examine the effect of polydispersity on the behavior of block copolymers in thin films. He is now working as a research associate with Prof. T. Davis in the Center for Advanced Macromolecular Design and with Dr. V. Bulmus in the School of Biotechnology and Biomolecular Sciences at UNSW.

processes that are thought to occur via the same mechanism and differ only in the structure of the compounds employed as chain transfer agents (coined RAFT agents). For the purposes of this review, both systems will be referred to as RAFT polymerization. RAFT agents are organic compounds possessing a thiocarbonylthio moiety. The generic structures of RAFT agents employed in RAFT and MADIX are shown below (Figure 1). The R group initiates the growth of polymeric chains, while the Z group activates the thiocarbonyl bond toward radical addition and then stabilizes the resultant adduct radical.

Jingquan Liu received his bachelor's degree from Shandong University, China, in 1989. His master's and Ph.D. degrees were obtained from the University of New South Wales (UNSW), Australia, in 1999 and 2004, respectively, where his Ph.D. was undertaken under the guidance of Professor Justin Gooding and Professor Michael Paddon-Row. In 2004 he worked as a CSIRO-UTS postdoctoral fellow prior to returning to UNSW, with Prof. Tom Davis as an UNSW Vice-Chancellor's Postdoctoral Research Fellow. He has coauthored over 31 peer-reviewed research papers. His research interests focus on the synthesis of various bio- and nanohybrids of versatile biodegradable and functional polymeric architectures.

Sébastien Perrier graduated from the Ecole National Supèrieure de Chimie de Montpellier, France, in 1998. He undertook his Ph.D. at the University of Warwick, England, in polymer chemistry and spent one year as a postdoctoral fellow at the University of New South Wales, Australia. In 2002, he was appointed as lecturer at the University of Leeds and was promoted to senior lecturer in 2005. In October 2007, he moved to the University of Sydney and was appointed as director of the Key Centre for Polymers & Colloids. Prof. Perrier leads a team of 10−15 researchers working at the interface of organic chemistry, polymer synthesis, and material science. He has published over 60 peer reviewed research papers and book chapters and over 50 conference papers/abstracts. Awards include an ARC international fellowship (2002) and the Macro Group U.K. Young Researcher Award (2006). His research interests lie at the interface of polymer synthesis and materials/soft matter science.

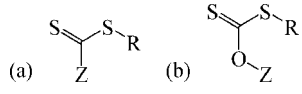


**Figure 1.** Generic structures of (a) the RAFT chain transfer agent and (b) the MADIX chain transfer agent ("RAFT agents").

### 1.1.1. Mechanism of RAFT

The generally accepted mechanism for a RAFT polymerization is shown in Figure 2. The first step of polymerization is the initiation step, where a radical is created (step

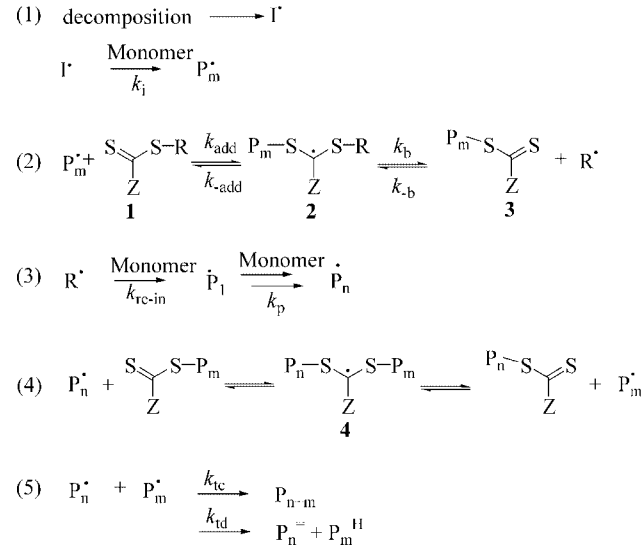


**Figure 2.** Generally accepted mechanism for a RAFT polymerization.

1). Many different sources of initiation have been reported for a RAFT polymerization, such as the thermal autoinitiation of monomers such as styrene,[8] direct photochemical stimulation of the CTA by ultraviolet light,[20,21] $\gamma$ radiation,[22−25] and pulsed laser irradiation.[26,27] The thermal decomposition of radical initiators is, however, the most widely adopted method of initiation, due to the commercial availability of such compounds.

The oligomeric radicals produced in the initiation step react with the RAFT agent (**1**) in a step of initialization (step 2). There is compelling evidence in the literature that all of the RAFT agents (if appropriately selected) are consumed in this step before any propagation commences.[28] This is due to the highly reactive C═S bond of the RAFT agent, which means that radical addition is favored over the addition to any of the double bonds that are present on the monomer. The radical intermediate (**2**) can fragment back to the original RAFT agent (**1**) and an oligomeric radical or fragment to yield an oligomeric RAFT agent (**3**) and a reinitiating R radical. The structure of R should be such that it is a good reinitiating group. It should fragment at least as quickly as the initiator or polymer chains from the stabilized radical intermediate (**2**). Following initialization, polymer chains grow by adding monomer (step 3), and they rapidly exchange between existing growing radicals (as in the propagation step) and the thiocarbonylthio group capped species (step 4, **4**). The rapid interchange in the chain transfer step ensures that the concentration of growing radical chains is kept lower than that of the stabilized radical intermediates (**4**), therefore limiting termination reactions. Although limited, termination reactions still occur *via* combination or disproportionation mechanisms (step 5).

### 1.1.2. Choice of RAFT Agents

The structures of the R and Z groups (Figure 1) are of critical importance to a successful RAFT polymerization. The R group of a RAFT agent is important in the pre-equilibrium stage of the polymerization. The R group should be a better leaving group than the propagating radical and must efficiently reinitiate monomer as an expelled radical. For certain monomers, such as MMA, the ability of a RAFT agent to effectively mediate the polymerization is highly dependent on the nature of the R group, whereas other

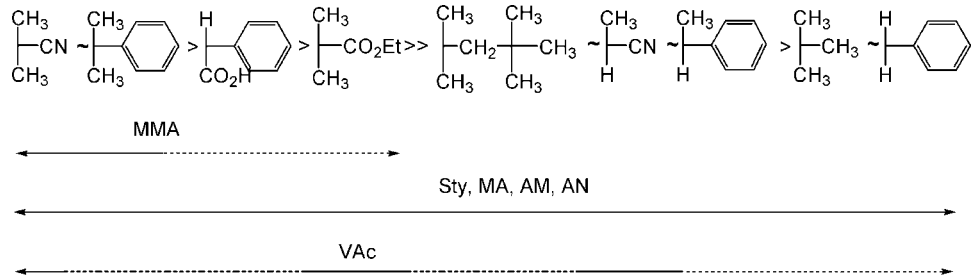



**Figure 3.** Guidelines for the selection of R group substituents for various polymerizations. Fragmentation rates decrease from left to right. Dashed lines indicate partial control over the polymerization (i.e., control over the molecular weight evolution but poor control over the PDI). MMA = methyl methacrylate, St = styrene, MA = methyl acrylate, AM = acrylamide, AN = acrylonitrile, VAc = vinyl acetate. Reprinted with permission from ref 91. Copyright 2005 CSIRO.

polymerization systems are more resilient with respect to the R group (Figure 3). Chong et al.[29] demonstrated the dependence of MMA polymerization on the nature of the R group. They investigated the performance of a range of dithiobenzoates in the polymerization of MMA and found that only the cumyl and cyanoisopropyl based R groups were able to efficiently reinitiate MMA monomer.

Steric factors, radical stability, and polar effects are significant in determining the leaving/reinitiating ability of an R group.[29,30] Increased radical stability enables the R group to be a good leaving group; however, if the radical is too stabilized, it may not effectively add onto a monomer and reinitiate polymerization. Increased steric bulk is likely to increase leaving group ability but is likely to have a detrimental effect on the reinitiating capability due to steric hindrance. Electron withdrawing substituents within the R group affect the electrophilicity of the derived radical. For instance, the cyano substituent of a cyanoisopropyl R group increases its affinity for electron rich vinyl groups, enhancing its ability to reinitiate monomer, thus making it an effective reinitiating group, despite its relatively high steric bulk. Design of RAFT agents so that the R group is structurally similar to the monomer being polymerized is occasionally employed. This allows the R group to have similar structural and electronic properties to the propagating radical, thus increasing reinitiation ability.

The Z group of a RAFT agent is highly influential in determining its reactivity and consequently its effectiveness at mediating polymerization. The Z group should be chosen so that it will activate the C=S bond toward radical addition and then impart minimal stabilization of the adduct radical formed.[31] If the stabilizing effect of the Z group is too high, fragmentation may not be favored and inhibition of the polymerization (in the initial step) or retardation (in the main process) might be observed. It is necessary to choose a Z group that is suitable for mediating the polymerization of a specific monomer. More reactive monomers are better controlled by RAFT agents that have a lesser activating effect on the thiocarbonyl group and, therefore, a greater destabilizing effect on the adduct radical, thus favoring fragmentation. The adduct radical formed by a more reactive monomer is more stable and less likely to undergo fragmentation. Thus, a Z group that destabilizes the adduct radical is required so that fragmentation can occur. An example case is the RAFT polymerization of vinyl acetate (VAc). VAc is a highly reactive monomer where the polymerization is only effectively mediated by xanthates[32,33] and dithiocarbamates,[34] which both cause destabilization of the adduct radical by virtue of their low chain transfer activity. Indeed, the lone pair electrons of the oxygen or nitrogen heteroatoms conjugate with the thiocarbonyl bond, thus reducing its double bond character and, hence, reducing its affinity for radical addition.[31] Upon radical addition to form an intermediate adduct radical, the lone pair electrons of the Z group heteroatoms induct toward the adduct radical, destabilizing it and, thus, promoting fragmentation.

For the same reasons that xanthates and dithiocarbamates are effective RAFT agents for mediating the polymerization of highly reactive monomers, they are not good for controlling the polymerization of less reactive monomers. For example, the polymerization of MMA is poorly controlled by xanthates[35] and dialkyl dithiocarbamates.[34] MMA radicals, which are less reactive due to greater stability, will not add efficiently to xanthate or dithiocarbamate thiocarbonyl bonds, which are not activated enough toward radical addition by their corresponding Z groups. The result is low rates of addition of monomer to the RAFT agent and high rates of fragmentation of the adduct radical. Consequently, the polymerization resembles a conventional FRP because the concentration of radicals in the system is too high. However, the activity of xanthates and dithiocarbamates can be "tuned" so that they become useful in the polymerizations of less reactive monomers. Mayadunne et al.[36] and Destarac et al.[34] found that the activity of dithiocarbamates could be increased by using a Z group where the lone pair electrons of the nitrogen are conjugated into an aromatic ring system or with a carbonyl group. Similarly, Moad et al.[30] and Destarac et al.[37] found that by introducing electron withdrawing groups into the alkoxy moiety of a xanthate, the activity of the thiocarbonyl group could be enhanced so that they can effectively mediate the polymerizations of less reactive monomers. Figure 4, suggested by the CSIRO group, can be used as a guideline for the selection of appropriate Z groups.[38]

### *1.1.3. Monomers*

Most of the monomers that are polymerized via conventional FRP can be polymerized with the RAFT methodology. This opens up the route to a wide range of functionality and makes the RAFT process the technique of choice to produce functional polymeric architectures. Styrene derivatives, acrylate and acrylamides, methacrylates, and methacrylamides and vinyl esters are typical classes of monomers used in RAFT polymerization.

Good to excellent control is achieved over the polymerization of styrene when mediated by dithioesters, trithiocarbonates, and dithiocarbamates (where the nitrogen lone pair does not strongly conjugate with the thiocarbonyl bond). However, the RAFT polymerization of styrene exhibits low

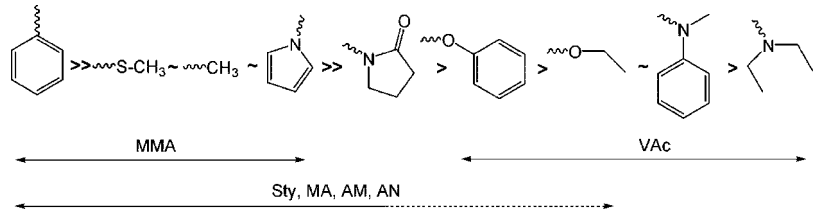



**Figure 4.** Guidelines for the selection of Z group substituents for various polymerizations. Fragmentation rates increase and addition rates decrease from left to right. Dashed lines indicate partial control over the polymerization (i.e., control over the molecular weight evolution but poor control over the PDI). MMA= methyl methacrylate, Sty = styrene, MA = methyl acrylate, AM = acrylamide, VAc = vinyl acetate. Reprinted with permission from ref 91. Copyright 2005 CSIRO.

rates of polymerization compared to most other monomers. Xanthates and alkyl dithiocarbamates generally offer poor control over the polymerization due to the low reactivity of the thiocarbonyl bond of these RAFT agents. The ability of xanthates and dithiocarbamates to mediate the polymerization of styrene can be improved by the inclusion of electron withdrawing groups attached to the alkoxy Z group[30,37] or, in the case of dithiocarbamates, where the nitrogen lone pair is conjugated into an aromatic ring system.[34,36,39]

Acrylates and acrylamides have also been widely studied, and their polymerization *via* RAFT usually leads to very well controlled polymers. Both monomers have a very reactive propagating radical with low steric bulk that leads to fast polymerizations, although an induction period is observed at the start of the polymerization.[40] The reasons for this have been reviewed elsewhere.[41] Polymerizations mediated by dithiocarbamates and most xanthates generally lead to broader molecular weight distributions (although living polymers are achieved with PDI between 1.2 and 2.3), while trithiocarbonates and dithioesters produce living polymers with low polydispersities (PDI ranges from 1.06 to 1.25). It is also noteworthy that the polymerization of acrylic acid is readily controlled by RAFT.[42−45]

Steric hindrance makes it difficult for the bulky tertiary propagating radical generated from methacrylate and methacrylamide derivatives to add to the C═S of the CTA. In order to favor addition to the thiocarbonyl bond, strongly stabilizing Z groups are required,[32] and dithiobenzoates are the best RAFT agents to control polymerization. Certain aliphatic dithioesters,[32] trithiocarbonates,[32] and dithiocarbamates[36] also lead to reasonably well controlled polymeric architectures (PDI ranging from 1.1 to 1.3), while xanthates, although producing living polymeric chains, offer very poor control. The R group also requires careful selection, as the stability of the generated radical (in order to favor preferential fragmentation with respect to that of the propagating polymeric radical) needs to be balanced with its reactivity, to favor addition to the monomer. For instance, using a reinitiating group that mimics the methacrylic propagating radical does not lead to narrow polydispersities, as the rate of fragmentation of the leaving radicals varies between a polymeric chain and a single molecule, due to the penultimate unit effect.[29] To date, there are only a few RAFT agents that produce polymers of methacryloyl derivatives with narrow molecular weight distribution. Cumyl dithiobenzoate[32] and cyanoisopropyl dithiobenzoate[32] are the best mediators for such polymerizations, while methoxycarbonylphenylmethyl dithiobenzoate[46] and α-cyanobenzyl dithioester[47] are the only RAFT agents with an R substituent generating a secondary radical that gives good control over methacrylic polymers.[48]

Vinyl acetate (VAc) is a relatively highly reactive monomer due to poor stabilization of the propagating radical and low steric bulk. RAFT polymerization is one of the rare polymerization processes that control the polymerization of vinyl esters. The lack of stability of VAc propagating radicals makes it a poor leaving group, which in turn means that the adduct radical is relatively stable. It follows that only RAFT agents, which strongly destabilize the adduct radical, such as xanthates[33] and *N*,*N*-dialkyl dithiocarbamates,[37] can effectively mediate the RAFT polymerization of VAc. The same is true for other monomers of relatively high reactivity, such as vinylpyrrolidone[49−51] and vinyl formamide, a precursor to prepare polyamine.[52]

A variety of other classes of monomers have been successfully polymerized by RAFT, including, for instance, isoprene,[53] 2- and 4-vinylpyridine,[54] acrylonitrile,[55] and allyl butyl ether (copolymerized with acrylates).[56] Specific monomers relevant to biological media are discussed in a later section of this review.

### *1.1.4. Polymer Functionality and Architecture*

Functionalities in RAFT polymers are not limited to the choice of monomers; they can also be introduced by polymeric chain end groups. RAFT polymers exhibit chain end functionalities, either introduced by the R or the Z group of the RAFT agent, or after *post*polymerization modifications. A number of techniques have been reported that demonstrate the modification of the thiocarbonylthio end group of a polymer prepared by RAFT.[29,47,57−68] Examples include the introduction of an olefin end group by thermal elimination of the thiocabonylthio group of a poly(methyl methacrylate),[69] the reduction of the dithioester end group into a thiol, followed by addition to a vinyl functional molecule,[59,64−66] or the simple addition of an excess of free radical initiators to replace the thioester end group by the radical generated from the initiator.[63] Further examples relevant to biological applications are discussed in a later section of this review.

RAFT is also a versatile tool for the engineering of complex polymeric architectures. The synthesis of a variety of copolymers, including random (statistical), gradient, alternating, graft, and block copolymers is easily achievable. Block copolymers of the type AB are one of the key products achievable via RAFT, and they are produced by sequential addition of a monomer B to a macro-RAFT agent produced by the polymerization of monomer A, mediated by the RAFT agent. The successful synthesis of well-defined polymers via this route requires careful consideration with respect to the sequence in which the monomers are polymerized. The propagating chain A must have a better, or comparable, leaving group ability with respect to monomer B. If A is a less stable radical than B, the adduct radical formed by attack of monomeric/polymeric radical B will preferentially fragment to release a propagating radical of B. Where monomer

reactivities are highly disparate, a controlled homopolymer of A and an uncontrolled homopolymer of B would be obtained.

Graft polymers are also easily achieved by RAFT polymerization. A graft copolymer is a type of branched polymer composed of a polymer backbone with polymer branches extending from the backbone. Graft copolymers can be prepared via RAFT polymerization in one of two ways: the "grafting from" technique[70] or the "grafting through" technique. The grafting through technique involves the polymerization of a polymeric chain showing reactive vinyl groups at its chain ends (a so-called macromonomer).[71] The "grafting from" technique involves the functionalization of a polymer backbone, or substrate, with a RAFT agent (either via its R group or via its Z group) or with a radical initiator.

Other more complex branched polymeric structures include highly branched polymers and star polymers. Highly branched polymers via RAFT have been designed as alternatives to dendrimers that are synthetically easier to achieve. These structures have been achieved either by self-condensing vinyl polymerization, using a RAFT agent that bears a polymerizable vinyl group,[72,73] or by polymerizing a monofucntional monomer in the presence of difunctional monomer and RAFT agent.[20,74,75]

Star polymers are structures that consist in linear polymeric chains that are joined by their end groups. There are two main strategies for synthesizing star polymers via RAFT: the core first approach and the arms first approach.[76] The core first approach requires the use of a multifunctional RAFT agent, where the polymer chains (arms) are grown from the core. The arms first approach involves the synthesis of polymeric arms of predetermined molecular weight, which are joined together *post*polymerization. Unique to the RAFT process, the core first technique can be performed in two ways. The thiocarbonylthio moiety can be attached to the core of the multifunctional RAFT agent through its Z group (Z group approach) or through its R group (R group approach).[76,77]

Significant termination reactions are often observed when employing the R group approach to star polymers. However, termination can be reduced in star polymer synthesis via the R group approach by using high temperatures, low number of arms, and high concentrations of RAFT agent compared to radical initiator, thus leading to more highly defined star polymers. The Z group approach would be expected to overcome many of the problems associated with the R group approach because the active center is immobilized on the core/multifunctional RAFT agent. This makes star−star coupling virtually impossible: no excess free linear chains are produced, and adjacent arms cannot terminate each other. Indeed, in most star syntheses via the Z group approach, termination reactions are negligible at low monomer conversions but increase significantly toward higher conversions.[78]

## 1.2. Application of the RAFT Technique to Biological Applications

Synthetic polymers and their hybrids with biological molecules have been increasingly used in biotechnology, biomedical, and pharmaceutical technologies since the mid-20th century. A few examples include pharmaceutical excipients, diagnostic components, biopurification matrices, and biomedical implants. Initially, macroscopic and microscopic properties were the major foci in designing polymers for biological applications. More recently, the perception of the role of nanoscale properties in applications has revealed opportunities for tailoring the properties of polymers at a molecular level to fulfill the performance criteria better for any given application.[79−87] While the design requirements can vary widely according to the application, the molecular weight, (supra)molecular architecture, composition, and chemical functionality appear to be the most important properties for a wide variety of applications, ranging from polymeric drug delivery systems to biocatalyst immobilized polymers. The uniformity in key properties, enabling performance to be correlated with structure, is usually desirable for most biological applications of polymers, such as polymer therapeutics and biomaterials surfaces.[80,84,88,89] The water-solubility or amphiphilic character of the polymeric systems is also essential for a number of applications, especially those involving biological molecules.

The RAFT polymerization approach offers a versatile platform for controlled synthesis and molecular engineering of vinyl polymers for biological applications. The major strengths of the RAFT approach include the following:

(1) An ability to control the polymerization of a wide range of monomers in varying solvents, including water, using only chain transfer agents and common free radical initiators (without the need for any additional polymerization component such as metal catalysts).[7,23,90−92]
(2) The tolerance to a wide variety of functional groups, allowing the facile synthesis of polymers with pendant, and alpha and omega end-group functionalities (an important feature for biological applications).[93−99]
(3) The ability to synthesize a wide variety of architectures such as telechelic, block copolymers, graft copolymers, gradient copolymers, nanogels, stars, and dendritic structures.[46,58,100−105]
(4) The compatibility of RAFT with a variety of established polymerization methods such as bulk, solution, suspension,[106] emulsion,[30,107,108] and dispersion[109,110] polymerizations.
(5) The ability to perform polymerizations from a wide variety of substrates, allowing the modification of surfaces and the in situ generation of polymer conjugates.[93,111−114]

Readers are also referred to other recent review articles that detail the strengths and different aspects of the RAFT technique when applied to various biological applications.[88,115−120]

## 1.3. Scope of the Review

This review compiles all the recent studies using RAFT polymerization for the design and the synthesis of polymers for bioapplications, such as gene/drug delivery, biomaterials, biomolecule−poymer conjugates, and hybrid organic/inorganic nanoparticles. This review highlights the advantages of RAFT polymerization, e.g. its great versatility and tolerance toward monomers, solvents, and temperatures for the deign of such materials.

# *2. Functional Polymers Obtained by RAFT Polymerization*

## 2.1. End-Group Functionalization

End-functional polymers can easily be designed via the judicious selection of RAFT agent structures. An α-functionality can be incorporated onto a polymer chain by

carefully designing the R group of a RAFT agent, while the $\omega$-end group of a polymeric chain can be controlled via modification of the Z group or by postmodification of the thiocarbonyl group after polymerization.

In what follows, we review publications that describe the design and use of functional RAFT agents for synthesizing polymers that could be applied to form bioconjugates. This section is divided into three parts:

(i) First, a general description of functional RAFT agents for the production of functional polymers is given.
(ii) Second, a description of functional RAFT agents useful for direct conjugation with (bio)compounds is given.
(iii) Finally, a description of biohybrid RAFT agents useful for in situ conjugation is given.

### 2.1.1. Functional RAFT Agents

The initial invention of RAFT polymerization has been followed by intense research activity to design novel functional RAFT agents for specific design purposes. Initially, this was primarily to allow optimum polymerization control over specific families of monomers. This activity has led to the description of various RAFT agent structures, such as trithiocarbonates, dithioesters, and xanthates. More recently, RAFT agent design has been stimulated by the motivation to control end-functional polymers, such as macromonomers or telechelic polymers.[103] A large range of functional RAFT agents has already been described in the polymer literature, including functionalities such as hydroxyl, carboxylic acid, and allyl, as listed in Table 1. Carboxylic acid is the most commonly used functionality used for making functional polymers by RAFT (Table 1). Lai et al.[121] reported the synthesis of several mono- and dicarboxyl functional RAFT agents, permitting control over a wide range of monomers, such as acrylate, acrylamide, and styrene, yielding monofunctional and telechelic polymers. Carboxyl functionalized trithiocarbonate or dithioester RAFT agents were developed for the polymerization of MMA by Moad et al.[61] and McCormick's team.[64,122−125] These carboxyl end-functional polymers can then be easily conjugated to peptides, proteins, or carbohydrates using traditional coupling chemistry approaches with either alcohol or amine groups. The carboxyl functionality can also be modified by chlorination, by pentafluorophenyl groups, by NHS, or by 2-mercaptothiozaline to further increase the yields of conjugation; for example, Aqil et al.[126] proposed a coupling of 5-([(*N*-biotinoylamino)hexanoyl]amino)pentylamine to a carboxylic acid functionalized poly(*N*-isopropyl acrylamide) (poly(NIPAAm)) using *N*-hydroxysuccinimide as an activator of the carboxylic acid group (Scheme 1). Aqil et al. reported an excellent coupling yield and the synthesis of biotin functionalized poly(NIPAAm), able to conjugate with avidin and strepavadin. However, this approach to bioconjugation has several drawbacks relating to the overall number of steps and the necessary purifications required. To negate these problems, activated ester RAFT agents were developed by several authors.[127−130]

Aamer and Tew[130] developed the synthesis of new activated ester RAFT agents by the modification of 4-cyanovaleric acid dithiobenzoate with NHS in the presence of DCC and DMAP. This RAFT agent exerted control over the polymerization of 4-vinylbenzoic acid (VBC), yielding low PDIs (<1.10); however, the experimental molecular weights were higher than targeted. This difference was attributed to the low efficiency of the RAFT agent. Xu and co-workers[129] described the synthesis of a dithioester bearing a mercaptothiazoline active ester able to control the polymerization of HPMA at 70 °C. The polydispersities remained below 1.2 during the polymerization, and the molecular weight of the growing polymer was linear, concomitant with monomer conversion. The 2-mercaptothiazolidine end group remained intact after the polymerization, yielding $\alpha$-mercaptothiazolidine terminated poly(HPMA). This group was then exploited to attach a dendrimer bearing four mannose groups. Recently, Theato and co-workers[131−133] described a RAFT agent and a diazoinitiator, both containing a pentafluorophenyl activated ester (PFP), to polymerize methyl methacrylate (MMA), diethylene glycol monomethyl ether methacrylate (DEG-MA), poly(ethylene glycol) monomethyl ether methacrylate (PEG-MA), hydroxyl propyl methacrylamide (HPMA), and lauryl methacrylate (LMA), giving homopolymers and diblock copolymers with control over molecular weight and narrow molecular weight distributions, at high conversions. Polymers derived from the PFP-RAFT approach possessed an $\alpha$-functionality that could be reacted with amines with high efficiency (close to 100%). Wiss et al. demonstrated the utility of the PFP-RAFT for the bioconjugation of polymer with a collagen peptide.[133]

RAFT agents bearing a hydroxyl group have also been studied to yield $\alpha$-hydroxyl and $\alpha,\omega$- hydroxyl terminated poly(MMA) or poly(*n*-BA) polymers with narrow molecular weight distributions (PDI < 1.3).[47]

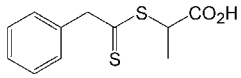
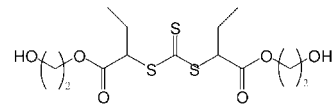
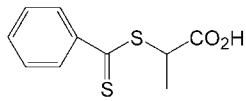
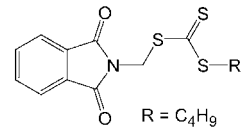
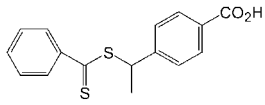
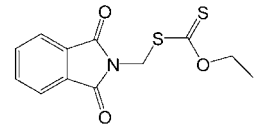
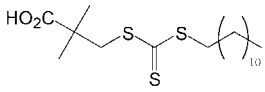
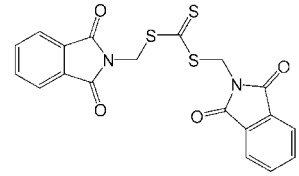
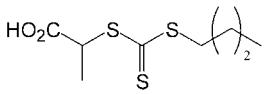
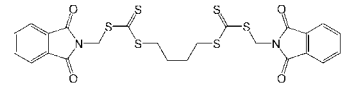
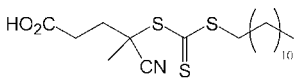
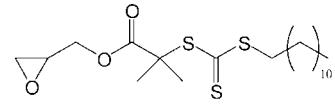
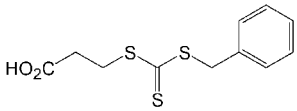
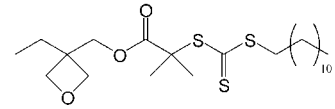
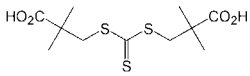
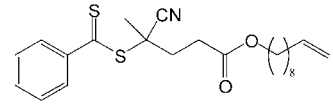
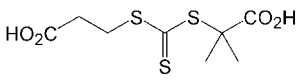
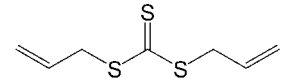
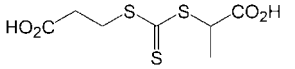
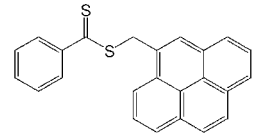
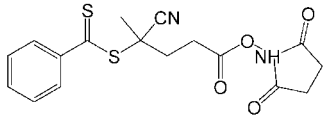
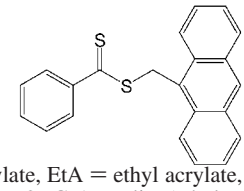
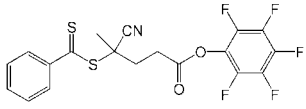
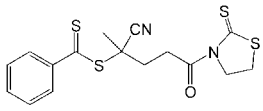
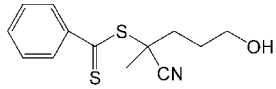
Vora et al.[134] proposed two new epoxy- and oxetane-functional RAFT agents able to control the polymerization of different acrylic monomers with PDIs below 1.1. The epoxy end group could be modified in the presence of different functionalities, such as amine and carboxylic acid, yielding macromonomers. The oxetane group was copolymerized in the presence of 3-ethyl-3-hydroxymethyl oxetane as a comonomer and $BF_3 \cdot (C_2H_5)_2O$ as a catalyst, yielding trithiocarbonylthio macromonomers. These epoxy and oxetane functionalities hold great promise for polymer bioconjugation applications, as they can be used in "click" type reactions with high efficiencies of reaction.

"Exotic" functional RAFT agents bearing $\alpha$-norbonenyl,[71] $\alpha$-allyl,[71] or $\alpha,\omega$-bis-allyl[135] and $\alpha$-cinnamyl[71] groups have also been described.[135] Allyl groups are of particular recent interest, as they can be exploited for modification via thiol−ene reactions, via UV exposure,[136,137] or in the presence of Karstedt's catalyst,[138] leading to more complex architectures. Maleimide terminated polymers were also obtained using a furan-protected maleimide chain transfer agent (CTA).[139] The furan protection was cleaved by heating the polymer at 110 °C to yield maleimide terminated poly(OEGA) with a functionality equal to 60−80%. Thiol functionalized lysozyne was conjugated to the polymer.

The synthesis of RAFT agents bearing fluorescence labels has also been reported with functionalization via a methyl anthracene R group[68] and a pyrenylmethyl.[140] Anthracene terminated RAFT agent yielded polymers of styrene and methyl acrylate with PDIs below 1.2. The resulting $\alpha$-fluorescence end labeled polymers exhibited enhanced fluorescence properties in chloroform and in DMF, for pyrenylmethyl and for methyl anthracene, respectively.

At the present time, primary and secondary amine and thiol functionalities are not directly accessible via RAFT agent design due to the degradation of the RAFT agent during the polymerization or the addition of monomers.[61] Several indirect routes have been proposed to overcome this limita-

**Table 1. Structures of Functional RAFT Agents**[a]

| Functions | RAFT agents | Monomers | Functions | RAFT agents | Monomers |
|---|---|---|---|---|---|
| α-carboxylic acid |  | NAM | α,ω-hydroxyl |  | MA, St, NIPAAm |
| α-carboxylic acid |  | NAM | α-amine |  | St, *n*-BA, NIPAAm |
| α-carboxylic acid |  | MMA, St. | α-amine |  | Vac, NVP |
| α-carboxylic acid |  | Et. A, AA, *n*-BA, HEA, *t*-BMA, St, MMA | α,ω-amine |  | NIPAAm, St, *n*-BA |
| α-carboxylic acid |  | AA, *n*-BA, NIPAAm | α,ω-amine |  | NIPAAm, St. *n*-BA |
| α-carboxylic acid |  | MMA | α-epoxy |  | St, Et A, *n*-BA, AA |
| α-carboxylic acid |  | St., NIPAAm, OEG-A, *n*-BA, 2-acryloyethyl phosphorylcholine, AA | α-oxetane |  | St, Et A, *n*-BA, AA, MA |
| α,ω-carboxylic acid |  | Et. A, AA, *n*-BA, HEA, *t*-BMA, St | α-allyl |  | St, MA, MMA |
| α,ω-carboxylic acid |  | MMA, *n*-BA | α,ω-allyl |  | St, *n*-BA |
| α,ω-carboxylic acid |  | *n*-BA | α- fluorescent |  | St |
| α-NHS |  | 4-vinylbenzoic acid | α- fluorescent |  | St, MA |
| α-pentafluorophenyl activated ester |  | NIPAAm, LMA, OEG-MA, DEG-MA, MMA | | | |
| α-mercapto-thiozaline activated ester |  | HPMA | | | |
| α-hydroxyl |  | MA, MMA | | | |

[a] Note: AA= acrylic acid, AM = acrylamide, AN = acrylonitrile, *n*-BA = *n*-butyl acrylate, EtA = ethyl acrylate, MA = methyl acrylate, MMA = methyl methacrylate, NIPAAm = *N*-isopropylacrylamide, NAM = *N*-acryloylmorpholine, OEG-A = oligo(ethylene glycol) acrylate, St = styrene, VAc = vinyl acetate.

tion, such as the protection of the amine group by the phthalimido group[96] or by a *tert*-butyloxycarbonate (*t*-Boc).[141] The amine group can be regenerated by deprotection in the presence of hydrazine and of trifluoroacetic acid (TFA) for phthalimido group and *t*-Boc, respectively.

### 2.1.2. RAFT Design Allowing a Direct Conjugation

In this section we describe functional polymers that can be directly and selectively conjugated without postmodification to biocompounds. A number of coupling reactions, such as thiol-pyridyl disulfide, thiol-maleimide, and alkyne-azide (click reactions), are established protocols for this direct conjugation approach (Table 2).

The pyridyl disulfide (PDS) functionality has been widely applied for bioconjugation[94,95,113,114,142−144] and for grafting of polymer onto gold surfaces[145] in recent years. PDS-thiol chemistry is selective, quick, versatile, and efficient.[142,146] The PDS group can be introduced via either R or Z groups

**Scheme 1. Postpolymerization Functionalization of Poly(NIPAAm) with Biotin via NHS-ester Activation**

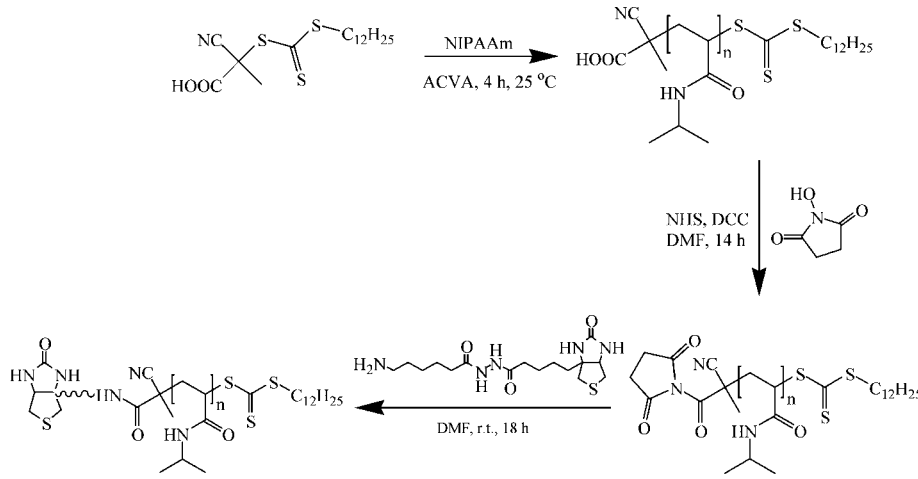
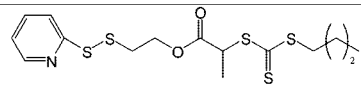
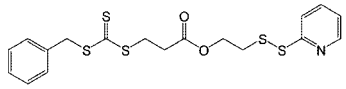
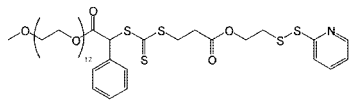
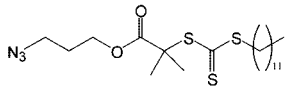
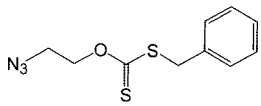
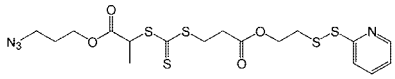



**Table 2. RAFT Agents Used for Direct Postpolymerization Conjugation[a]**

| Functions | RAFT agents | Monomers |
|---|---|---|
| α-pyridyl disulfide |  | OEG-A |
| ω-pyridyl disulfide |  | OEG-A, St., NIPAAm |
| ω-pyridyl disulfide |  | OEG-A, NIPAAm |
| α-azide |  | NIPAAm |
| α-azidc |  | VAc |
| α-azidc, ω-pyridyl |  | St., NIPAAm |

[a] Note: AA = acrylic acid, AM = acrylamide, AN = acrylonitrile, *n*-BA = *n*-butyl acrylate, EtA = ethyl acrylate, MA = methyl acrylate, MMA = methyl methacrylate, NIPAAm = *N*-isopropylacrylamide, NAM = *N*-acryloylmorpholine, OEG-A = oligo(ethylene glycol) acrylate, St = styrene, VAc = vinyl acetate.

(or R and Z together), to yield telechelic polymers with narrow PDIs, for example, monofunctional pyridyl disulfide functionalized poly(styrene),[147] poly(OEG-A),[95,143] and poly(NIPAAm).[143] The presence of pyridyl ethyl disulfide bonds does not affect the RAFT mechanism for fast monomers (i.e., (meth)acrylic and acrylamide).[143] The PDS group can be used to conjugate with thiol functionalities present in biomolecules, such as bovine serum albumin (BSA),[94,114,148] peptide (for example, NGR[93] or glutathione peptide[147]), and small interfering RNA[144] (siRNA) with high yields generating protein/peptide/RNA polymer conjugates or micelles. Moreover, the conjugation occurs via a disulfide bond that can be readily reduced *in vivo* to permit the release of the biocompound from the synthetic polymer.[149]

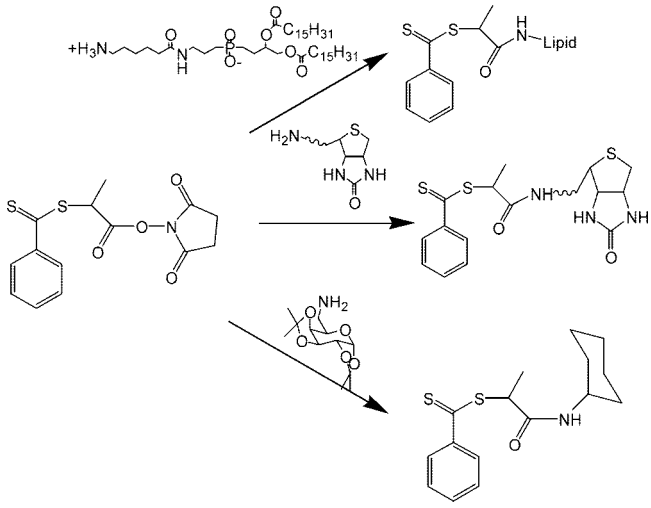
Several authors have proposed the synthesis of azide-functional RAFT agents for application in "click" type conjugation approaches. Using "click" chemistry,[150] it is possible to graft polymers onto alkyne modified bovine serum albumin (BSA) with high yields, as described by Li et al.[151] In a similar approach, the CAMD team[94] proposed the use of a heterotelechelic α-azide, ω-pyridyl disulfide functional RAFT agent able to couple with alkyne modified biotin by a click reaction (95% efficiency). The use of this functional RAFT agent yielded heterotelechelic poly(NIPAAm) and poly(styrene), that could be conjugated to different proteins, i.e. BSA and avidin. Chen and co-workers[152] described the synthesis of azide modified RAFT agents for the polymerization of vinyl acetate at 80 °C. However, control over vinyl acetate is often problematic and PDI was seen to increase with conversion. In their paper, Chen et al.[152] attributed the broadening to transfer to monomer and polymer during the polymerization. However, Ladmiral et al.[153] have described some problems with the use of "click" functionality in certain polymerizations at higher temperatures, and Favier et al.[154] have also noted the potential strong influence of impurities in RAFT polymerization. After polymerization of vinyl acetate, Chen et al.[152] attached fluorescent labels (propinyloxy coumarin) using click chemistry (catalyst system: CuBr/PMDETA), obtaining high yields.

### 2.1.3. Synthesis of Biohybrid RAFT Agents

Bathfield and co-workers[127,128] have described a new RAFT agent bearing an activated ester in the R group, i.e. for the direct attachment to biomolecules (Scheme 2). This succinimidyl ester readily reacts with nucleophilic groups (such as amine) in a one-step reaction. Bathfield et al. demonstrated that the amidation reaction was favored over the thioamidation (aminolysis) to yield a RAFT agent bearing an amide bond. This reaction protocol was expanded to attach different compounds, such as sugar (galactose), *N*-aminoethylmorpholine,[127] and, recently, a phospholipid.[128] The rapid reaction of amine onto *N*-hydrosuccimide avoids any degradation problems, i.e. aminolysis of the RAFT agent. However, it is important to use a $[RAFT]_0/[amine]_0$ ratio superior to (or equal) to 1 to avoid aminolysis side reactions.

A biotin modified RAFT agent was synthesized by Hong and Pan,[155] coupling biotinylated alcohol and *S*-1-dodecyl-*S*′-(α,α′-dimethyl acetic acid) in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP). This RAFT agent exerted control of NIPAAm and *N*-(2-hydroxypropylacrylamide) (HPMA) with a PDI range of 1.09−1.20 and, also, allowed the synthesis of poly[(NIPAAm)-*block*-(HPMA)] diblock polymer.

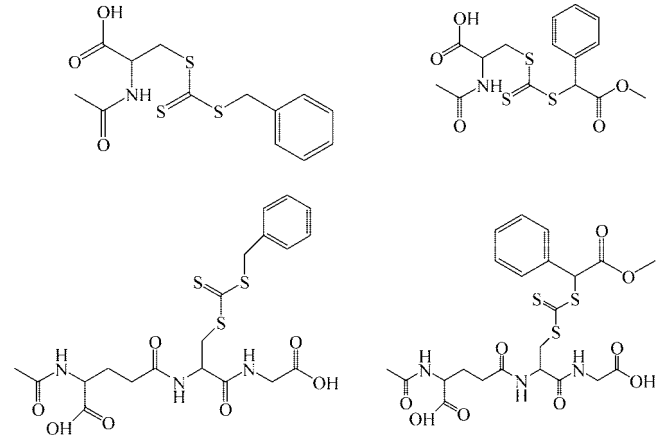
**Scheme 2. Chemical Modification of the Ester Activated RAFT Agent by Biocompounds, Such as Phospholipid, Biotin, and Carbohydrate**



Boerner and co-workers[156−159] expanded the RAFT polymerization technique to the synthesis of well-defined peptide−polymer conjugates. An original approach was developed via solid-phase supported synthesis, making unnecessary any purification by silica chromatography. One approach involved the attachment of peptide via reaction of an amine terminated supported peptide onto carboxylic acid functionalized RAFT agent in the presence of a catalyst for the coupling reaction between amine and acid (DCC/EDC, DMAP). However, this approach is hindered by the nucleophilic attack of peptide amine onto the dithioester (aminolysis). RAFT agent was separated from the support by a dilute TFA/DCM solution (2%) and obtained with 76% purity. It is interesting to note that TFA does not affect the integrity of the RAFT agent; however, some *tert*-butyl ester groups were lost during the workup. The second route involved the modification of a peptide bearing bromine or chlorine atom (ATRP initiator) by nucleophilic substitution with a pyridinium salt of the dithiobenzoic acid in THF, yielding an oligopeptide macro transfer agent. These macroRAFT agents exerted polymerization control of *n*-BA at 60 °C. After *in situ* polymerization, the chilarity of peptide was preserved (demonstrated by circular dichroism analysis).

Zhao and Perrier[160] used peptides bearing a cysteine to prepare peptide macroRAFT agents (Scheme 3). To illustrate this example, they synthesized four different peptide-macroRAFT agents with high yield (95%) in methanol (Scheme 4). These RAFT agents exerted polymerization control for

**Scheme 4. Different Peptide-MacroRAFT Agents**



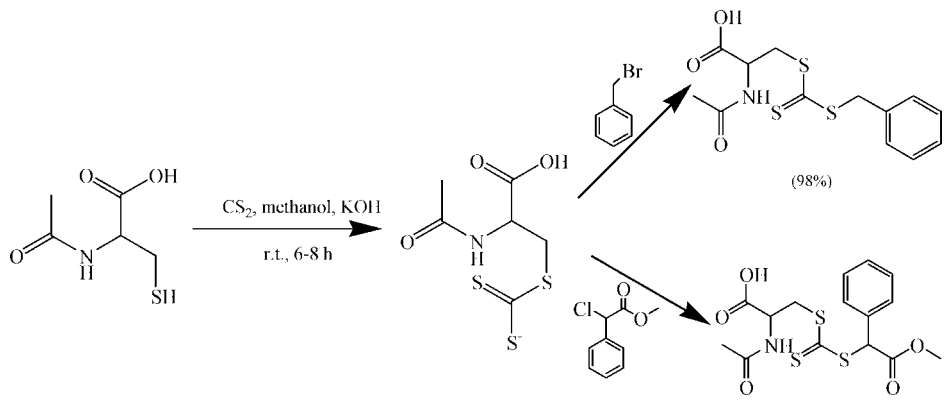
a number of monomers (NIPAAm, dimethyl acrylamide (DMA), *n*-BA, and methyl acrylate). This synthetic approach is useful for simple peptides; however, it has some limitations. It cannot be applied to complex peptides for two majors reasons: first, the experimental conditions of RAFT group modification may alter the peptide structure (chilarity), and, second, any peptide bearing free amines cannot be modified by this process, as it is necessary to protect the free amine, as reported by Zhao and Perrier,[160] during the synthesis and the polymerization. Subsequently, the amine needs to be protected by a *t*-Boc group.

The synthesis of a BSA−macroRAFT agent using the selective reaction of thiol-pyridyl disulfide was proposed by the CAMD team[113,114] to generate BSA−poly(NIPAAm) and BSA−poly(OEG-A) conjugates. The attachment of a thermosensitive polymer, poly(NIPAAm), allowed regulation of the BSA activity and the design of nanoparticles (<200 nm).[113] De et al.[161] reported the attachment of BSA using an R approach (Scheme 5), with thiol−maleimide coupling. The resultant BSA−macroRAFT agent was used to control the polymerization of NIPAAm at room temperature using similar conditions to those reported by Boyer and coworkers.[113] Disulfides present in native BSA were also reduced to increase the number of free thiols per protein, thereby providing multiple attachment sites per BSA. The R approach reduces steric hindrance that can in some cases (Z approach) reduce the polymerization efficiency.

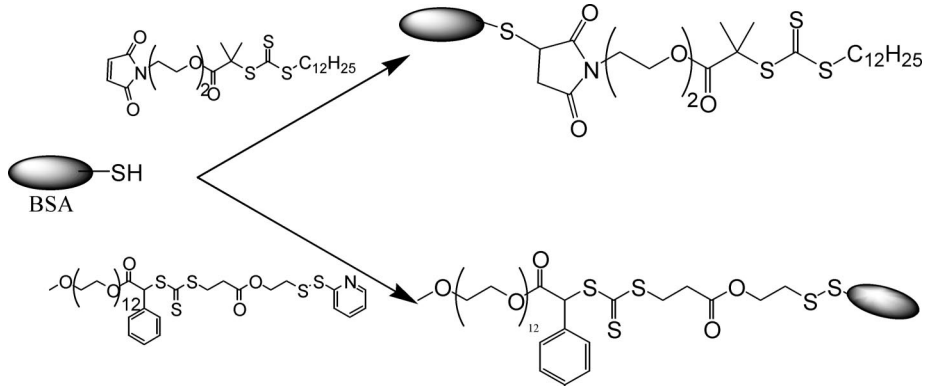
### 2.1.4. Chemical Modification of a RAFT End Group

An alternative to RAFT polymer functionalization is the direct modification of RAFT functionality to generate reactive end groups suitable for conjugation to biomolecules.

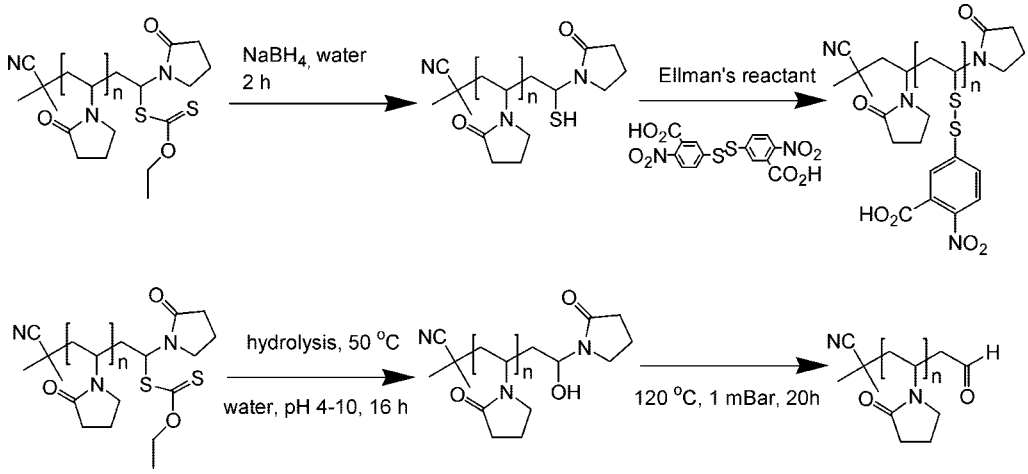
**Scheme 3. Synthesis of RAFT Agents Using Free Thiol**



**Scheme 5. Synthesis of BSA−MacroRAFT Agent by Two Different Routes: Thiol−Maleimide Addition and Thiol−Pyridyl Disulfide Exchange**




**Scheme 6. Chemical Modification of Poly(NVP) Obtained by RAFT Polymerization: (top) Reduction of the RAFT End Group by $NaBH_4$ in the Presence of Ellman's Reagent To Yield a Protected Thiol; (bottom) Hydrolysis of the RAFT End Group to Yield an Aldehyde End Group**




There are several possibilities, including aminolysis of the RAFT agent into thiol or radical modifications. This route presents the advantage to reduce the toxicity of these polymers by removal of the RAFT end group.[162]

Recently, Pound and co-workers[163] presented a route to modify poly(vinylpyrrolidone) by hydrolysis of RAFT end groups at pH 4.5, yielding hydroxyl end groups (Scheme 6). These hydroxyl groups were then transformed, via thermolysis, into aldehyde groups. Aldehyde functionality can react easily with amine end groups on proteins, peptides, or oligonucleotide biomolecules. To illustrate this reaction, the authors conjugated the amine groups of lysine with poly(vinylpyrrolidone) to lead to lysozyne−poly(vinylipyrrolidone) conjugates, with a good yield. However, no bioactivity data was reported, and so it was unclear whether the conjugates had any potential utility.

Zelikin and co-workers[164] modified the xanthate terminii of PVP chains generating thiol end groups, that were protected by reaction with Ellman's reactant to yield disulfides. This approach facilitates the use of versatile thiol chemistry, such as thiol−maleimide or thiol−disulfide exchange, to introduce reactive end groups such as fluorescent labels, peptides, or oligonucleotides.

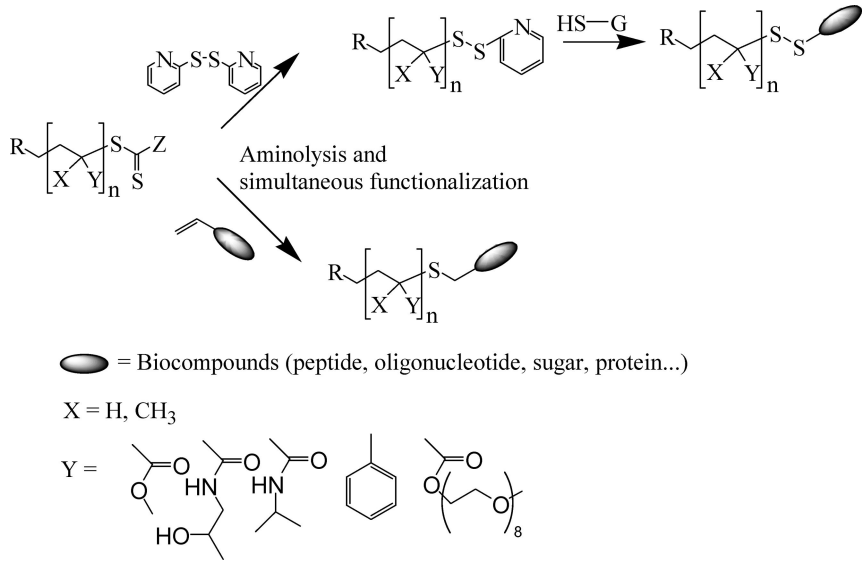
Several authors[47,58,61,62,97,165−172] proposed the modification of RAFT end group terminated poly(NIPAAm) to generate thiols, with subsequent bioconjugation via thiol−ene reactions.

For example, You and Oupick[172] proposed a two-step strategy: first, a thiol functionalized poly(NIPAAm) was obtained by degradation of trithiocarbonate in the presence of hexylamine under nitrogen. Second, thiol terminated poly(NIPAAm) was reacted to 1-biotinamido-4-[4′-(maleimidomethyl)cyclohexanecarboxamido]butane. In this process, some byproducts were observed, such as that of disulfide interchain coupling.

Convertine et al.[90] successfully used this route to conjugate poly(NIPAAm) with fluorescein functionalized maleimide. To avoid the formation of disulfide interchain coupling, the addition of tri-*n*-butyl phosphine as a reductant was proposed. Accordingly, the disulfide interchain coupling was totally eliminated.

Thiol−ene addition was also exploited for the synthesis of homo- and heterotelechelic polymers.[47] Recently, An and co-workers[173] proposed another methodology involving a cascade aminolysis/Michael addition and alkyne-azide click reactions to generate well-defined heterofunctional polymeric materials. This methodology is very similar to Qui and co-workers' approach.[168] First, RAFT agent was reduced into thiol and then reacted to form a fluorescein o-acrylate, followed by the modification of azide groups in the presence of a dansyl probe via click chemistry using a $CuSO_4 \cdot 5H_2O$/sodium ascorbate catalyst system to obtain α-fluorescein, ω-dansyl poly(NIPAAm). In Qui's approach,[168] the reduction of RAFT agent terminated poly(NIPAAm) was used to add alkyne groups by addition of thiol onto alkyne acrylate monomers, followed by a click reaction between azide and alkyne to lead to cyclic polymers. This approach was reported to work with high efficiency. Yu and co-workers used a similar approach to synthesize alkyne terminated pol(NIPAAm);

**Scheme 7. Chemical Modification of Polymers Obtained by RAFT Polymerization: (top) Reduction of the RAFT End Group in the Presence of 2,2-Dithiodipyridine To Yield Pyridyl Disulfide Terminated Polymers, Followed by Bioconjugation Using Thiol Terminated Compounds; (bottom) Aminolysis of the RAFT End Group in the Presence of an Ene Group, Such as Carbohydrate Methacrylate or Maleimide Modified Biotin**




however, in a second step, the authors proposed to click two thiols on the alkyne group using a thiol-yne reaction.[174]

Recently, Li and co-workers[169] proposed the use a bismaleimide to react with thiol functionalized poly(NIPAAm) obtained by reduction of RAFT end groups to give a maleimide functionalized poly(NIPAAm) with a high yield. To ensure the absence of interchain coupling, a large excess of bismaleimide was used. The maleimide end group can then be exploited to add another thiol compound by thiol−ene addition. The nucleophilic thiol obtained after aminolysis of the RAFT end-group can also be exploited for other reactions such as thiol−isocyanate, as described by Li et al.[174b] who used this reaction for the functionalization of poly(diethyl acrylamide).

The CAMD team[175] developed two original approaches using the thiol generated from RAFT aminolysis (Scheme 7). First, aminolysis in the presence of 2,2′-dithiopyridyl disulfide (DTP) was carried out to generate pyridyl disulfide end groups.[176] Second, the aminolysis of the RAFT agent was carried out in the presence of functional enes, such as biotin functionalized maleimide, sugar modified methacrylate, or di(meth)acrylate compounds to lead to new macromonomer types[177] by thiol−ene addition. These two routes produced different functional polymers with good yields (close to 90%) without accompanying side reactions such as disulfide or thiolactone formation. Kakwere and Perrier followed a similar approach to attach biotin to the shell of soft nanoparticles (particle sizes around 30−40 nm) made from RAFT polymers, followed by complexation to avidin.[59]

RAFT end group removal and functionalization was also reported using radical addition.[63] This approach utilizes a large excess of radicals generated by initiators (such as azo compounds) at the end of the polymerization (in the absence of monomers), leading to the formation of polymeric chain radicals, which can recombine irreversibly with one of the free radicals present in excess in solution, thus forming a dead polymer chain. This method eliminates the RAFT end groups and also introduces new functionality at the of the polymer chain ends. Thus, new functional groups are introduced according to the type of initiator and the chain transfer agent can be recycled simultaneously. For example, Roth and co-workers[131] proposed this strategy to remove terminal $\omega$-dithioester groups of polymer chains while using a pentafluorophenyl ester diazo compound to functionalize RAFT polymers with a PFP ester at the $\omega$-end. As a consequence, functionalization of both end groups was possible, leading to telechelic polymers, exhibiting an active ester at both ends of the polymer chain. Another recent example was proposed by Heredia and co-workers[148] to yield a heterotelechelic poly(NIPAAm), with one chain end bearing biotin. A maleimide was introduced to the $\omega$ chain-end by reaction via a radical cross-coupling reaction with a functionalized azo-initiator. Telechelic biotin-maleimide poly(*N*-isopropylacrylamide) was used for the formation of streptavidin−bovine serum albumin (BSA) polymer conjugates. A similar methodology was employed to yield BSA telechelic poly(NIPAAm).[178] Maynard's group[178b] described the synthesized of four-arm protein−poly(NIPAAm) conjugated using thiol−maleimide coupling reactions.

Another approach has also been developed to enable functionalization of RAFT polymers using the monoaddition of maleimide monomers. N-substituted maleimido monomers have been used in a modified block polymerization to add a single maleimido unit onto the RAFT polymer with nearly quantitative efficiency. This technique has been demonstrated using *N*-(2-aminoethyl)maleimide trifluoroacetate introducting a single primary amine to the $\omega$-terminus of poly(DMAEMA) and poly(*NIPAAm*) and to a specialized block copolymer for siRNA delivery. This chemistry was exploited to construct diblock copolymers with a bioconjugation site located precisely at the block junction. The chain-extended polymers were then functionalized with an amine-reactive fluorescent dye or folic acid with conjugation efficiencies of 86 and 94%, respectively.[178c]

## 2.2. Pendant Groups Functionalization

RAFT[61,91] polymerization is a versatile radical polymerization technique for the inclusion of functional monomers, together with ATRP[11,12,19] and iodine transfer polymerization.[4] In what follows, we report the more useful monomers utilized for bioconjugations (Figure 5).

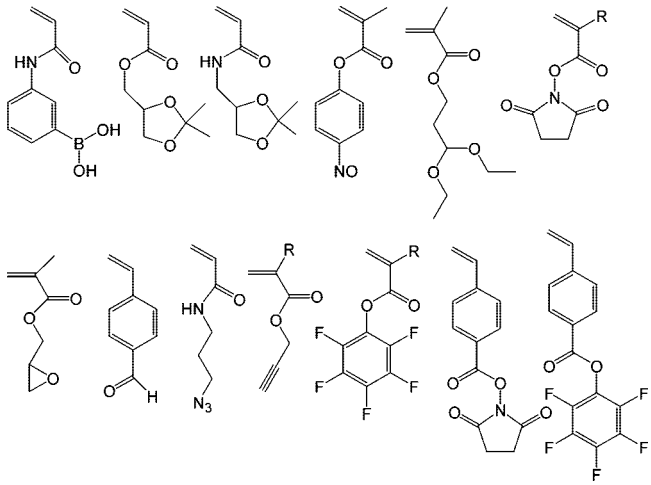
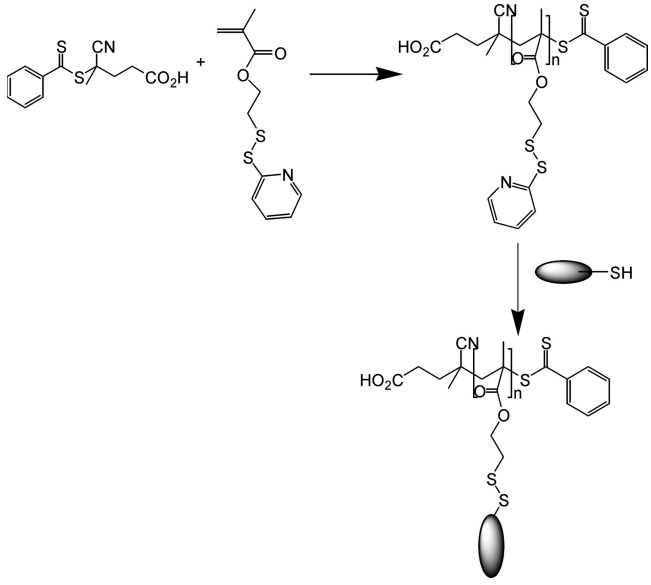


**Figure 5.** Functional monomers suitable for bioconjugation (note: R corresponds to H or $CH_3$).

**Scheme 8. Polymerization of Pyridyl Ethyl Disulfide Methacrylate Monomer by RAFT Polymerization, and the Chemical Modification by Thiol Compounds**



### 2.2.1. Thiol Reactive Monomers

Pyridyl disulfide ethyl methacrylate was successfully homopolymerized for the first time by RAFT polymerization by Bulmus, Davis, and co-workers using CPAD (Scheme 8).[98,104] Control of PDI and molecular weight was achieved in DMAc, as the solvent, and AIBN, as the initiator, at 70 °C. The presence of disulfide bonds did not affect the polymerization, with no significant transfer observed for fast monomers.[143] The presence of pyridyl disulfide as a pendant group creates a scaffold amenable to modification by thiol compounds, such as 2-mercaptopropionic acid, 2-mercaptoethanol, or glutathione.[98] In addition, the pyridyl disulfide can be cleaved in the presence of TCEP to yield free thiols suitable for reacting onto maleimide compounds, such as doxorubicin modified maleimide.[104] This monomer was also copolymerized by RAFT in the presence of HPMA and OEG-MA to obtain poly(PDSM-*block*-HPMA)[104] and poly(PDSM-*block*-OEG-MA) diblock and random polymers with a PDI less than 1.2.

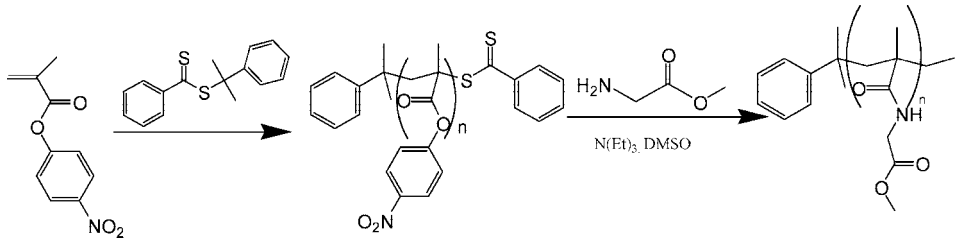
Allyl pendant groups can be introduced using a large range of monomers, as suggested by Ma and co-workers[179] for RAFT polymerization and by Campos et al.[136] and Strandwitz et al.[137] for ATRP. These allyl groups can be easily modified by thiol−ene reactions in the presence of thermal or UV initiators, leading to functional polymers, such as glucose polymers.[180] Valade et al. proposed the synthesis of block copolymers of allyl methacrylate and *N*-(2-hydroxypropyl-)methacrylamide by RAFT polymerization (PDI < 1.4).[181] The allyl group was modified with cysteamine compound via thiol−ene reaction, with a very high efficiency (∼100%), yielding cationic copolymers. These copolymers were used for the complexation of siRNA.

### 2.2.2. Activated Ester Monomers

The activated ester monomers constitute an important class of bioapplicable compounds. One of the most studied is *N*-acryloxysuccimide (NHS-A). NHS-A can yield a polymer bearing succinimidyl-activated ester pendant groups. NHS-A was successfully homopolymerized[182] and copolymerized by RAFT with NIPAAm,[183] *N*,*N*-demethyll acrylamide (DMA),[184] and *N*-acryloylmorpholine (NAM)[185] to give water-soluble random copolymers (using both dithioesters and trithiocarbonates). By working at the azeotropic composition (60/40: NAM/NHS-A in mol %), polymer chains without composition drift were obtained.[186] RAFT copolymerization of NHS-A has been extended to other architectures, such as block copolymers in the presence of DMA, NAM, and *tert*-butyl acrylate (*t*-BA), to yield the hydrophilic block copolymer poly(DMA-*block*-NHS-A) and the amphiphilic block copolymers poly[*t*-BA-*block*-(NHS-A-*co*-NAM)].[186] Li and co-workers[187] used this monomer in the presence of a macroRAFT agent bearing a PEO block to obtain poly(ethylene oxide)-*block*-poly(DMA-*co*-NHS-A) diblock polymers or poly(ethylene oxide)-*block*-poly(DMA-*co*-NHS-A)-*block*-poly(NIPAAm) triblock polymers. These polymer precursors were used for several applications, such as the attachment of peptide, DNA, or fluorescent dye.[188−190] The NHS-A units were also modified in the presence of ethylene diamine, spermine, or *N*,*N*-dimethyl ethylene diamine to yield RAFT polymers bearing primary or secondary amine pendant groups. The methacrylate analogue to NHS-A, *N*-methacryloxysuccimide (NHS-MA), has also been polymerized by RAFT. However, it appeared difficult to control the homopolymerization (broad polydispersity was observed), while its copolymerization in the presence of NIPAAm[183,191] or HPMA[192] allowed improved control, yielding different copolymers with a range of 2% to 30% NHS-MA. Poly(HPMA-*co*-NHS-MA) was modified by a peptide yielding a polymer capable of complexation to anthrax.[192]

Another activated ester monomer type, pentafluorophenyl (meth)acrylate, has been used for (bio)applications.[193] These monomers were homopolymerized or copolymerized using RAFT polymerization.[194,195] For example, the synthesis of functional amphiphilic poly(pentafluorophenyl methacrylate)-*block*-poly(lauryl methacrylate) copolymers was performed in the presence of 4-cyano-4-(thiobenzoyl)sulfanylpentanoic acid. Block copolymers with molecular weights from 12 000 to 28 000 g/mol and PDIs of about 1.2 were obtained. The pentafluorophenyl methacrylate was modified in the presence of hydroxyl propyl amine to generate poly(HPMA)-*block*-poly(lauryl methacrylate) or in the presence of a fluorescent dye (4-nitro-7-(piperazin-1-yl)benzo[*c*][1,2,5]oxadiazole (NBD), yielding diblock copolymers bearing fluorescent pendant groups.[132] Gibson and co-workers[195b] explored the feasibility of using poly(pentafluorophenyl methacrylate) as

**Scheme 9. Synthesis of Polymers with Reactive Pendant Groups Using *p*-Nitrophenyl Methacrylate (NPMA) Functionality in the Presence of Cumyl Dithiobenzoate (CDB) as a CTA and AIBN as an Initiator**




a reactive polymeric precursor to synthesize a diverse polymer library via post-polymerization modification, with nine different amines yielding polymethacrylamides at high yields. This postpolymerization modification approach failed to induce any additional cytotoxicity, making it an ideal approach to bioactive-polymer libraries. The CAMD team[195c] also exploited these activated ester monomers for the synthesis of glycopolymers using different amine functionalized carbohydrates (such as glucosamine, galactose amine, etc.), obtaining polymers at high yields (superior to 95%), while simultaneously subjecting the RAFT end-groups to aminolysis/thiol−ene reactions with biotin modified maleimide yielding in one pot biotin funtionalized glycopolymer able to bind with streptadivin or axidin. These polymers (i.e., poly(pentafluorophenyl acrylates) and poly(pentafluorophenyl methacrylates)) exhibit better reactivity and solubility in organic solvents than the corresponding poly(NHS-A) or poly(NHS-MA).[193]

Hwang and co-workers[196] proposed the synthesis of polymers with reactive pendant groups using *p*-nitrophenyl methacrylate (NPMA) by RAFT polymerization utilizing cumyl dithiobenzoate (CDB) as the CTA (Scheme 9). The activated ester polymers from the RAFT polymerization were subsequently modified by an amino compound, i.e. a glycine methyl ester, with yields of 86%.

Recent interest in 4-vinylbenzoic acid derivatives has extended the pool of pendant polymeric activated esters. Aamer and Tew[130] described the RAFT polymerization of the *N*-succinimide activated ester of 4-vinylbenzoic acid (NHS-VB), leading to poly(NHS-VB) with a low PDI (<1.07) and controlled molecular weights. An improved solubility of this monomer compared to NHS-A or NHS-MA probably assists in the excellent RAFT polymerization results. Another monomer, pentafluorophenyl ester 4-vinylbenzoic acid, was utilized by Nilles and Theato,[197,198] yielding polymers with solubility in a number of organic solvents.

### 2.2.3. Amine Functionalized Monomers

Amine groups can be introduced on polymer backbones using different monomers, such as *N*,*N*′′-(dimethylamino-)ethyl methacrylate,[199−202] 2-aminoethyl methacrylamide hydrochloride,[99,203,204] and *N*-vinylphthalimide,[205] via RAFT polymerization. In the case of 2-aminoethyl methacrylamide, the polymerization was carried out in a mixture of water/dioxane or in an acetate buffer[99] using the protonated form to avoid side reactions, i.e. Michael addition of amine onto the methacrylate bond[141,206] and degradation of the RAFT agent by aminolysis.[48] Different architectures were reported such as homopolymer, diblock copolymer, and random copolymer. The presence of primary amine on the backbone facilitates the conjugation of biomolecules, such as folic acid.[204] In the case of tertiary amine, several monomers can be used and copolymerized with different comonomers, such as NIPAAm. The presence of cationic charge can be used to complex Si-RNA, as described by Scales et al.[166] In the case of *N*-vinylphthamide, the polymerization was successful using different xanthates. A correlation of theoretical and experimental molecular weights was reported with PDIs below 1.5. After polymerization, the primary amine can be regenerated by deprotection in the presence of hydrazine to lead to poly(vinyl amine).[205]

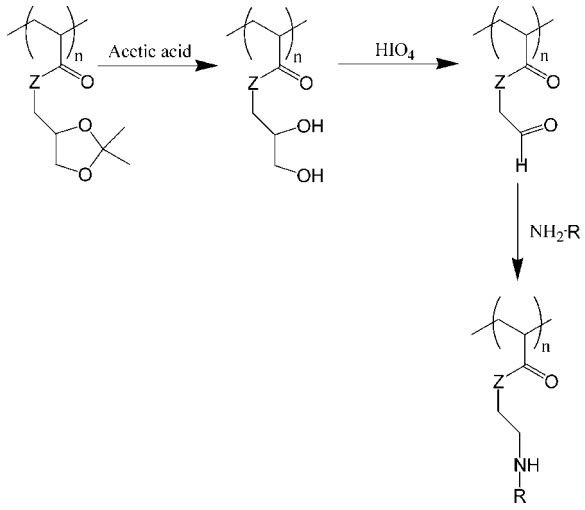
### 2.2.4. "Clickable" Monomers

Azide or alkyne monomers have been polymerized by RAFT agents to yield copolymers bearing azide or alkyne pendant groups. Zhang and co-workers[207] copolymerized propargyl methacrylate (PMA) and oligo(ethylene glycol) methacrylate (OEG-MA) in the presence of 4-cyanopentanoic acid dithiobenzoate (CPAD) at 60 °C to give poly(OEG-MA-*block*-PMA) diblock copolymers with a PDI below 1.2. It is interesting to note that the alkyne groups were reported to remain benign in RAFT polymerization. After polymerization, a pyrene modified azide was clicked in the presence of CuCl and PMDETA, as the catalyst system, to produce a polymer bearing pendant pyrene groups. Azide modified (meth)acrylate was also polymerized and copolymerized using RAFT. Jiang and co-workers[208] obtained double hydrophilic diblock copolymers, poly(*N*,*N*-dimethylacrylamide)-*block*-poly(*N*-isopropylacrylamide-*co*-3-azidopropylacrylamide) (poly(DMA)-*block*-poly(NIPAM-*co*-AzPAM), with low PDIs (PDI < 1.3). After polymerization, the authors proposed to cross-link by click chemistry using a telechelic alkyl to obtain micelles. In contrast, Li and co-workers[209] reported the synthesis of polymers with higher PDIs (>1.4) when the polymerization was carried out at a high temperature for a long time.

### 2.2.5. "Unusual" Monomers

Sumerlin and co-workers[210,211] used a boronic acid monomer to introduce pendant groups in polymer chains. The approach was based on the polymerization of 3-acrylamidophenylboronic acid (APDA) and *N*,*N*′′-dimethyl acrylamide (DMA) in the presence of 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid to yield poly(APDA-*block*-DMA) diblock copolymers. The synthesis of APDA homopolymer was achieved with a PDI < 1.2. The boronic acid group can be exploited, as it is pH sensitive and can complex with diol. According to the pH of the solution, boronic acid will be soluble (pH > p$K_a$) or insoluble (pH < p$K_a$) in water. Another important class of reactive monomers that can be (co)polymerized in the presence of RAFT agent are monomers bearing aldehyde groups. Aldehyde functionality is widely used for (bio)conjugation to amine groups within peptides and proteins. The copolymerization of poly(ethylene glycol) methyl ether methacrylate (OEG-MA)

**Scheme 10. Chemical Modification of (2,2-Dimethyl-1,3-dioxolane)methyl acrylate (DDMA) Obtained by RAFT Polymerization To Yield Polymers with Aldehyde Pendant Groups by Modification in the Presence of Acetic Acid and Periodic Acid**




with one of two dioxolane-containing monomers, (2,2-dimethyl-1,3-dioxolane)methyl acrylate (DDMA) and (2,2-dimethyl-1,3-dioxolane)methyl acrylamide (DDMAA), was reported using RAFT polymerization[212] in dimethylformamide (DMF). The resultant copolymers had narrow molecular weight distributions (PDI typically between 1.2 and 1.3), while monomer conversions were typically 60%. A kinetic study revealed that OEG-MA was consumed at a higher rate than that for the comonomers, implying that the copolymerization reactivity ratios of OEG-MA ($r_1 \approx 1$) are larger than those for the comonomers, DDMA ($r_2 \approx 0.43$) and DDMAA ($r_2 \approx 0$). After copolymerization, the dioxolane functional groups were deprotected to form 1,2-diol groups and subsequently oxidized with periodic acid ($HIO_4$) to form reactive aldehyde groups (Scheme 10). Subsequent chemical modification of the dioxolane to aldehyde groups occurred in the absence of any polymer degradation. The availability of backbone aldehyde groups for conjugation with amine containing molecules was confirmed by reaction with the iron chelating drug, desferrioxamine (DFO). Sun and coworkers[213] proposed the synthesis of another monomer, i.e. 4-vinylbenzaldehyde (VBA), and presented a successful RAFT polymerization in the presence of *S*-1-dodecyl-*S*-(α,α′-dimethyl-α″-acetic acid) trithiocarbonate, as a chain transfer agent. Excellent control of molecular weights and PDI was reported (PDI < 1.2). The synthesis of poly((VBA)-*block*-poly(styrene)) diblock copolymers was also achieved with a low PDI (<1.2). Sun et al. proposed the synthesis of amphiphilic block copolymer poly$(EO)_{45}$-*block*-poly$(VBA)_{26}$ and their self-assembled to yield vesicule with a size centered at 250 nm.[214]

A new aldehyde-functional glycomonomer, 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose (IVDG), was designed and prepared by Xiao and coworkers.[215] The "living" radical polymerization of IVDG was successfully achieved using AIBN as the initiator and 1-phenylethyl dithiobenzoate as the RAFT agent at 60 °C in THF. The molecular weights increased with monomer conversion, and the molecular weight distribution was narrow (PDI < 1.1). The protective isopropylidene groups from the sugar residue in polyIVDG were removed quantitatively using 88% formic acid at room temperature, yielding a novel amphiphilic polymer containing both galactopyranose and aldehyde functionalities. These amphiphilic polymers were shown to self-assemble into well-defined aldehyde-bearing polymeric micelles in aqueous solution in the absence of surfactant. Protein-bioconjugated nanoparticles (<200 nm) were also successfully prepared by immobilization of BSA (as a model protein) onto the aldehyde functionalized micelles.

Hwang and co-workers[196] proposed the synthesis of polymers with diethoxypropyl methacrylate (DEPMA) by RAFT polymerization, utilizing cumyl dithiobenzoate (CDB) as CTA and AIBN as initiator (Scheme 11). Narrow molecular weight distributions (PDI < 1.3) were obtained at high monomer conversions (86%). The side chains of poly(DEPMA) were hydrolyzed to aldehyde groups and reacted with *O*-benzylhydroxylamine and *O*-methylhydroxylamine to form stable oxime bond conjugates. The degree of substitution was dependent on the feed ratios. Subsequently, conjugation to a model peptide, i.e. an aminooxy functionalized RGD peptide, was demonstrated.

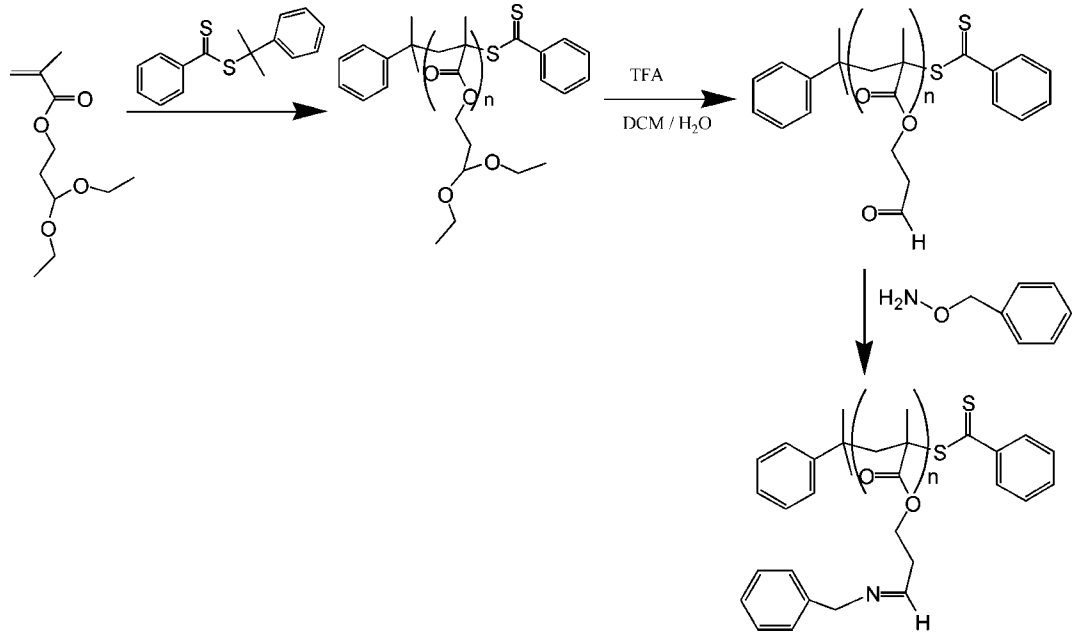
Another important reactive functionality used for the modification of polymers is the epoxy group.[216,217] The successful RAFT polymerization of glycidyl methacrylate (GMA) has been demonstrated by two research groups. The results show that GMA can be successfully polymerized and hydrolyzed to form a hydrophilic glyceryl functionality.

## 3. Bioconjugates

Bioconjugates of polymers have attracted increasing interest as a result of their extensive applications in medicine, biotechnology, and nanotechnology.[86,218,219] Attachment of polymers to biomolecules can mediate their stability, solubility, and biocompatibility. Bioconjugation to synthetic molecules can also impart additional functionality to the biomolecules, thereby inducing novel self-assembly, patterning, and phase behavior. For example, when proteins are attached to poly(ethylene glycol) (PEG) chains (PEGylation), their *in vivo* blood circulation times can be significantly increased. PEGylation can also increase the protein stability *in vivo*, by decreasing the biomolecules' vulnerability to proteolytic enzymes and antibodies.[220] There are two general approaches taken to form bioconjugates; postpolymerization conjugation of functionalized polymers to biomolecules and the *in situ* polymerization of monomers directly at a site on the biomolecule. The postpolymerization conjugation approach usually necessitates complicated multistep purification processes, resulting in lower yields. In addition, other problems can affect postpolymerization conjugation, such as nonspecific absorption and multisite attachment. In contrast, the *in situ* polymerization approach appears advantageous with regard to purification, attachment specificity, synthesis, controllability, and yield. However, problems may still occur with sensitive biomolecules under normal polymerization conditions. Postpolymerization conjugation methods require synthetic polymers with functional terminal groups. These functional groups can be introduced either during the polymerization process or via postpolymerization modification, as explained in the sections above. The *in situ* polymerization method utilizes biomolecules modified with a polymerization controlling agent (initiator or transfer agent), followed by polymerization.

The increasing utility of polymer conjugates of proteins in medicine,[89,218,220−226] biotechnology,[227−232] and nanotechnology[219,221,232−236] has driven research into generating ho-

**Scheme 11. Synthesis of Polymers with Aldehyde Pendant Groups from Diethoxypropyl Methacrylate (DEPMA) Utilizing Cumyl Dithiobenzoate (CDB) as a CTA and AIBN as an Initiator and Modification with an Amino-Oxy Compound**




mogeneous and well-defined conjugates manifesting uniformity in biohybrid properties and consistent reproducible biological activity. Living radical polymerization has become a popular synthetic methodology, as it facilitates reduced polymer heterogeneity and easy polymer end-group control. Nitroxide mediated polymerization,[1,2,82,237,238] atom transfer radical polymerization (ATRP),[11,19,239−245] and reversible addition−fragmentation chain transfer polymerization (RAFT)[7,46,61,91,125,127,147,155,246−248] techniques have been used for the synthesis of well-defined end-group-functionalized polymers that can be directly conjugated to proteins without the need for postpolymerization end-group modifications. All polymerization approaches have advantages and disadvantages, with RAFT favored by many, with the claimed advantages of solvent and functionality tolerance and the absence of any metal ions. However, ATRP has many strong advocates who maintain that copper removal is facile and effective.[249] Beside, a highly active copper based catalyst for ATRP was recently developed, allowing a considerable reduction of copper.[13,14,250,251] Another method allowing an important reduction of copper in the polymers is the single electron transfer−living radical polymerization (SET-LRP)[15] and the single electron transfer−degenerative transfer living radical polymerization (SET-DTLRP)[6,252−254] developed by Percec. Both SET-LRP and SET-DTLRP allow the control of a large range of monomers, such as methyl acrylate,[254−258] methyl methacrylate,[15] and vinyl chloride.[15,259] To avoid the presence of copper, which is toxic, several authors proposed to replace copper by another metal,[19] such iron,[260,261] ruthenium,[262,263] etc.

Other advantages for the RAFT approach have been cited as the use of common radical initiators and the low toxicity of some RAFT agents (although more work is required in this area). In some cases, ATRP requires the use of sacrificial initiators (seen as a disadvantage), although recent developments refining the ATRP approach may make some of the criticisms of ATRP obsolete.[264] Provided the RAFT polymerization protocols are optimized, then the ratios of monomer to RAFT agent[38,46,61,64,90,101,265−268] are higher than the ratios of monomer to initiator used in many ATRP-controlled polymerizations,[11,269−271] negating the requirement for large quantities of modified proteins to control RAFT polymerizations.

## 3.1. Fabrication of Protein−Polymer Conjugates

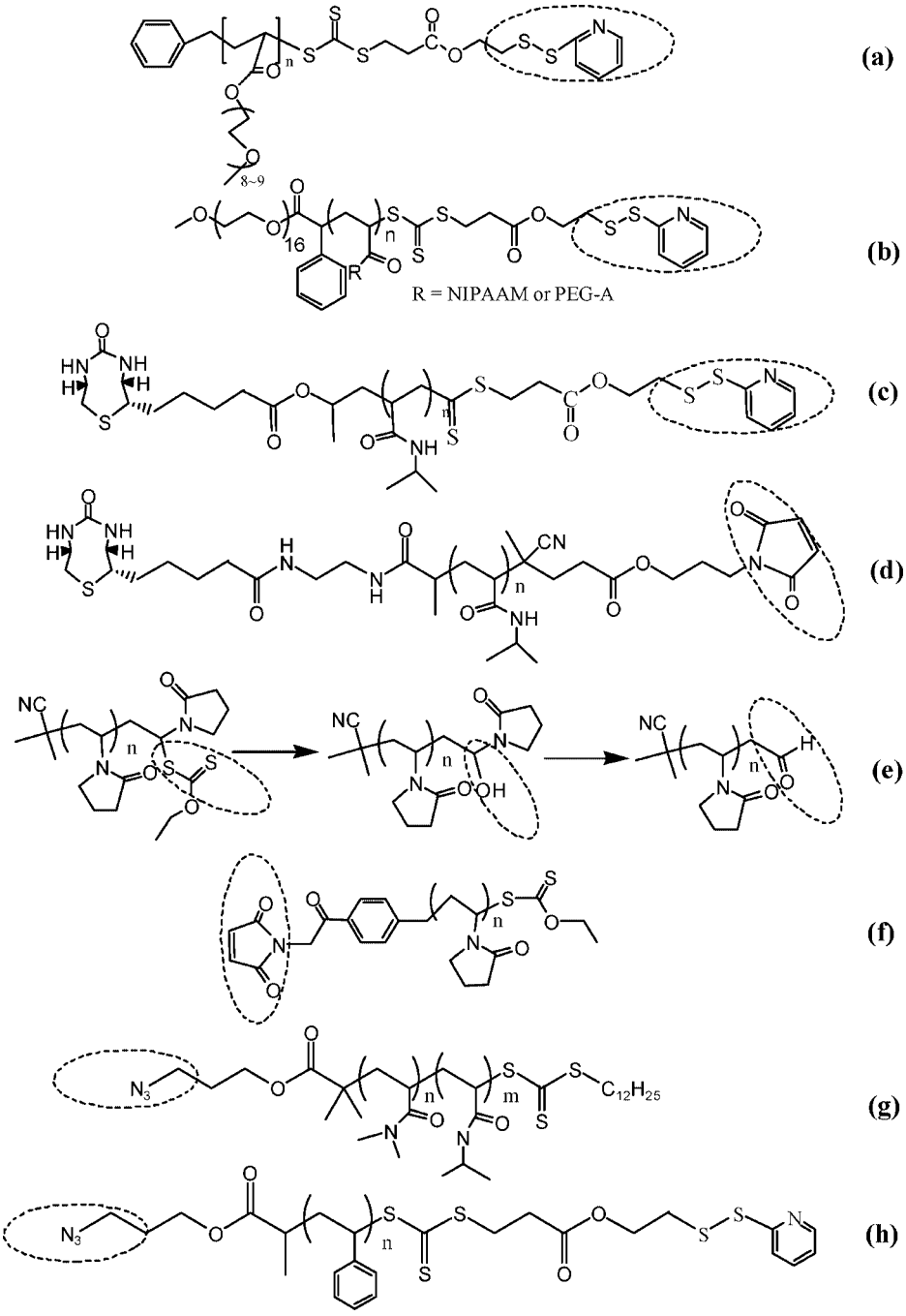
### *3.1.1. Postpolymerization Conjugation Methodology*

Studies on postpolymerization conjugations using RAFT polymers have generally focused on the modification of the RAFT end group as a linker. In many cases, aminolysis or reduction of the thiocarbonylthio has been employed,[97,246,272,273] yielding thiol-ended polymers.[97,272,273] Ideally, it would be advantageous to avoid any postpolymerization modification. A recent review by Heredia and Maynard[231] collated data on functionalized polymers (mainly synthesized using ATRP) for conjugation to proteins. In this current review we focus on RAFT polymerization as an approach to the design and synthesis of bioconjugates as summarized in Scheme 12.

**3.1.1.1. Conjugation via a Protein's Thiol Groups.** Boyer et al.[143] reported two RAFT agents functionalized with a PDS group and suitable for inducing living radical polymerization (Scheme 12a and b). RAFT agents were shown to be effective over the temperature range 25−70 °C. Successful RAFT polymerizations were demonstrated for the polymerization of NIPAAm and olig(ethylene glycol)-acrylate (OEG-A) in both water and acetonitrile. The kinetic data indicated that the PDS functionality is largely benign in free radical polymerizations, remaining intact for subsequent reaction with thiol groups. The PDS terminated polymers were successfully attached to BSA, as evidenced by GPC and polyacrylamide gel electrophoresis (PAGE) analyses.[143]

The same group[94] extended their work to the synthesis of more complicated heterotelechelic protein−polymer conjugates. In this study biotinylated polymers with PDS thiol-reactive terminal groups were synthesized, followed by a site-specific attachment of BSA via a cleavable disulfide linkage. These polymers were then conjugated with avidin, yielding heterotelechelic α-avidin, ω-BSA-polymer conjugates. This

**Scheme 12. RAFT Polymers Functionalized for Conjugation to Biomolecules**



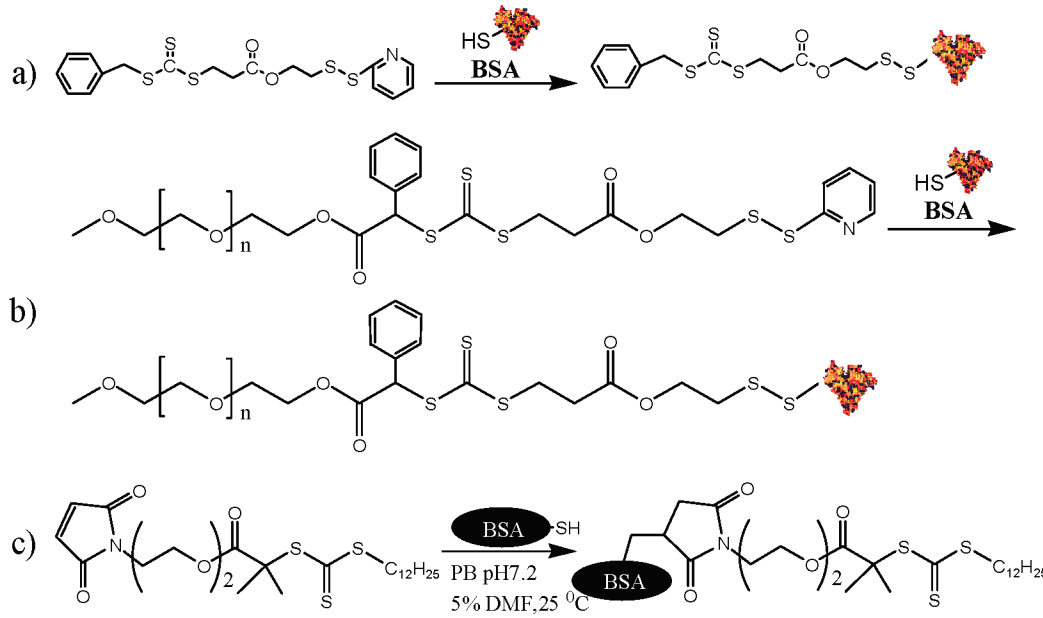

methodology can be easily extended to heterotelechelic polymer bioconjugates of other biomolecules (Scheme 12c).

Heredia and co-workers[148] reported the successful synthesis of heterotelechelic polymers with biotin and maleimide groups, as shown in Scheme 12d. The maleimide functionality was used to attach free-thiol tethered BSA via maleimide−thiol chemistry, and the other end was used to bind streptavidin via an affinity interaction.

**3.1.1.2. Conjugation via a Protein's Amine Groups.** ATRP has been adopted for making aldehyde terminal polymers;[241] similarly, RAFT polymerization was adopted to synthesize $\omega$-aldehyde poly(*N*-vinylpyrrolidone) via the quantitative conversion of xanthate-ended functional precursors, followed by the conjugation of the aldehyde-ended polymer to lysozyme using amino groups present in the protein (an unstable linkage under basic conditions[163]) (Scheme 12e). Xiao et al.[215] have also successfully attached proteins on a micellar surface with aldehyde groups via oxime coupling.

In a study by McDowall[274] and co-workers, lysozyme conjugated with seven poly(*N*-vinylpyrrolidone) (PVP) arms was synthesized by conjugating linear *N*-succinimidyl ester terminated PVP polymer to lysozyme amino groups. The polymerization of *N*-vinylpyrrolidone proceeded in a living fashion up to more than 90% conversion, reaching molecular weight of up to 33 000 g/mol with narrow molecular weight distributions (Scheme 12f).[274] In a more recent work, Tao et al.[275] synthesized lysozyme−polymer conjugates using thiazolidine-2-thine coupling chemistry and investigated the bioactivity of the conjugates. It was found that the molecular weight of the polymer and the pH of the coupling reaction

**Scheme 13. Preparation of Protein-MacroRAFT Agents for Fabrication of Protein−Polymer Conjugates via *in Situ* Polymerization**




were significant factors in optimizing the subsequent bioactivity of the conjugates.

**3.1.1.3. Conjugation via "Click Chemistry"** Li and coworkers[151] adopted copper-catalyzed azide−alkyne click chemisty to synthesize responsive protein−polymer conjugates. In their study, BSA was functionalized with an alkyne moiety via reaction of its free cysteine residue with propargyl maleimide. Azido terminated poly(NIPAAm) was prepared via RAFT, and the protein−polymer coupling was accomplished by copper-catalyzed azide−alkyne cycloaddition (Scheme 12g).

Alkyne−azide click chemistry was also utilized by the CAMD team[94] for bioconjugations. A series of heterotelechelic polymers with an azido and a pyridyl disulfide (PDS) group on chain terminii were synthesized. Subsequently, the azido group was subjected to a click reaction with alkyne modified biotin (Scheme 12h).[94]

### *3.1.2. In Situ Polymerization Methodology*

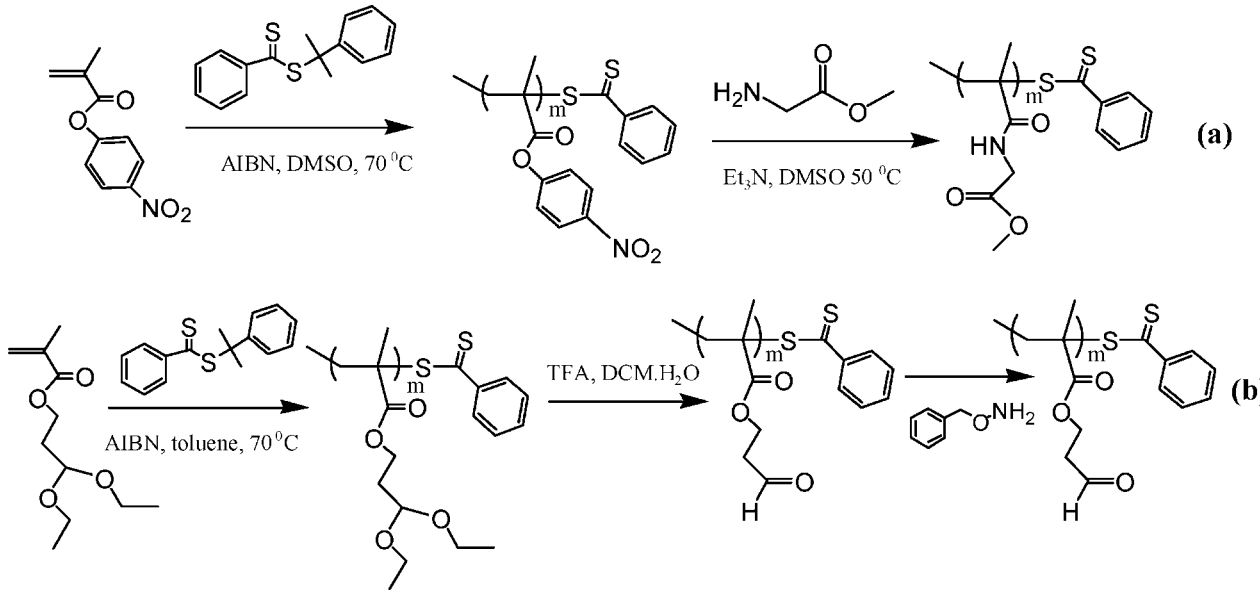
The in situ approach to conjugate formation has many advantages, as detailed earlier. Pioneers in this research area were Maynard,[231,245,276,277] Haddleton,[117] and Russell.[278] In these pioneering studies, and also in a more recent study by Le Droumaguet and Velonia,[279,280] proteins were first modified with ATRP initiator(s) at a defined site, e.g. cysteine residues of BSA[276,281] and mutant lysoszyme,[276,281] and lysine residues of chymotrypsin.[278] Polymerizations were then performed from the ATRP initiating sites of proteins in the presence or absence of a sacrificial initiator to form protein−polymer conjugates *in situ*. Subsequently, the CAMD team[114] successfully synthesized polymer−protein conjugates via *in situ* RAFT polymerization using a BSA-RAFT macroRAFT agent obtained from site-specific modification of BSA at its cysteine 34 residue with a thiol-reactive RAFT agent via disulfide coupling. The subsequent *in situ* polymerization of poly(PEG-A) via $\gamma$-radiation at room temperature afforded well-defined BSA−polymer conjugates that retained 92% bioactivity. The nondenaturing PAGE of the dialyzed polymerization mixtures confirmed the formation of polymer conjugates. Control experiments indicated that the *in situ* polymerization only occurred at the site of RAFT agent attachment (Scheme 13a). The CAMD team[113] further optimized the *in situ* polymerization conditions by synthesizing water-soluble PEG-RAFT agents. The attachment of the water-soluble RAFT agent to BSA afforded a completely water-soluble BSA−macro-RAFT agent that was then used to control the polymerization of two different water-soluble monomers: NIPAAm and hydroxyethyl acrylate (HEA), at ambient temperature. The growth of the polymer chains from BSA−macroRAFT agent was confirmed by size exclusion chromatography (SEC), $^1$H NMR, MALDI-ToF, and polyacrylamide gel electrophoresis (PAGE) analyses (Scheme 13b).

BSA-RAFT agent was also synthesized by De *and coworkers*[161] using thiol-maleimide coupling chemistry, followed by the *in situ* polymerization of Poly(NIPAAm) to form thermally responsive polymer-protein conjugates (Scheme 13c).

## 3.2. Biotinylated Polymers for Conjugation to (Strept)avidin

Biotin, vitamin H or $B_7$, is a water-soluble B-complex vitamin and is necessary for cell growth, production of fatty acids, and the metabolism of fats and amino acids. Biotin binds very tightly to the tetrameric protein avidin (also streptavidin and neutravidin), with a dissociation constant $K_d$ on the order of $10^{-15}$ mol/L, which is the strongest known protein−ligand interaction, approaching the covalent bond in strength. A variety of applications in biotechnology exploit the affinity bonding between biotin and streptavidin, e.g. bioseparations and surface patterning *via* self-assembly. Biotin has a valeric acid "tail", through which it can be modified with other precursors (biotinylation). Biotinylated polymers have been synthesized for selectively binding with streptavidin.[126,282,283] Biotinylation of polymers can be achieved either by postpolymerization conjugation[247,284] or via straightforward generation of biotinylated polymer and diblock copolymers using a biotinylated RAFT agent.[155,283] A biotinylated RAFT chain transfer agent was synthesized using

**Scheme 14. RAFT Controlled Polymerization of (a) *p*-Nitrophenyl Methacrylate (NPMA), with Subsequent Coupling with Amino Acid, and of (b) Diethoxypropyl Methacrylate (DEPMA), with Deprotection and Coupling to RGD Peptide**




the esterification reaction of a carboxylic acid terminated trithocarbonate RAFT agent with a biotinylated alcohol. Direct polymerization using AIBN initiation generated homo- and block polymers, suitable for streptavidin conjugation.[155] Particles can also be modified with biotinylated (co)polymers for surface bioconjugation to streptavidin.[126,282] Narain et al.[282] have successfully modified magnetic nanoparticles (iron oxide, size inferior to 100 nm) using monodisperse biotinylated poly(NIPAAm) with subsequent conjugation to streptavidin for potential applications in magnetic resonance imaging, drug delivery, biosensors, and hyperthermia treatment of cancer. Well-defined glycopolymers containing linear and cyclic carbohydrate moieties as pendant groups were also prepared by RAFT polymerization for aqueous synthesis of stabilized glyconanoparticles with surface bound biotin termini for bioconjugation to streptavidin.[285] The biotinylation of nanoparticles has been accompanied by modification with glycopolymers in order to elevate their biocompatibility.[283,284]

Biotinylated polymers can also be synthesized using postpolymerization modification methods. In a study by You and Oupicky,[172] the temperature−responsive heterobifunctional block copolymers of PEG and poly(NIPAAm) were first synthesized via RAFT polymerization, followed by the aminolysis of the RAFT end groups to free thiols, for conjugation to maleimide modified biotin. A similar approach was adopted by Kakwere and Perrier to modify the surface of soft nanoparticles (size around 30−40 nm) produced from RAFT polymers.[59]

## 3.3. Peptide−Polymer Conjugates

Peptides are information-rich molecules, with many biomedical applications;[286] however, they are not stable in the body. An approach to obviate this problem is the attachment of biocompatible polymers, e.g. PEG and HPMA fragments, to form conjugates with higher stability and molecular size, over the excretion threshold. A set of peptide−polymer conjugates were synthesized using RAFT polymerization from peptide modified RAFT agents.[156,287] These peptide conjugates can self-assemble into fibrillar microstructures and left-handed superhelical fine structures via a pH controlled rearrangement.[156,287] Hwang and co-workers[196] have also reported the synthesis of polymers of *p*-nitrophenyl methacrylate (NPMA) and diethoxypropyl methacrylate (DEPMA) utilizing cumyl dithiobenzoate (CDB) as the RAFT agent and azobisisobutyronitrile (AIBN) as the initiator. As shown in Scheme 14, the *p*-nitrophenyl methacrylate (NPMA) pendant groups on the polymer backbone can be modified to aldehyde through which amino-oxy terminated RGD precursor can be coupled via oxime bonding. Zhao and Perrier[160] also prepared peptide modified RAFT agents that were used for direct preparation of peptide terminated polymer conjugates. Boyer and co-workers[175] have also reported polymer conjugates of a hexapeptide (GNGRGC) with a pyridyldisulfide terminated poly(NIPAAm), generated via RAFT polymerization.

Micelles can also be decorated with peptides using RAFT chemistry, as shown in Scheme 15.[147]

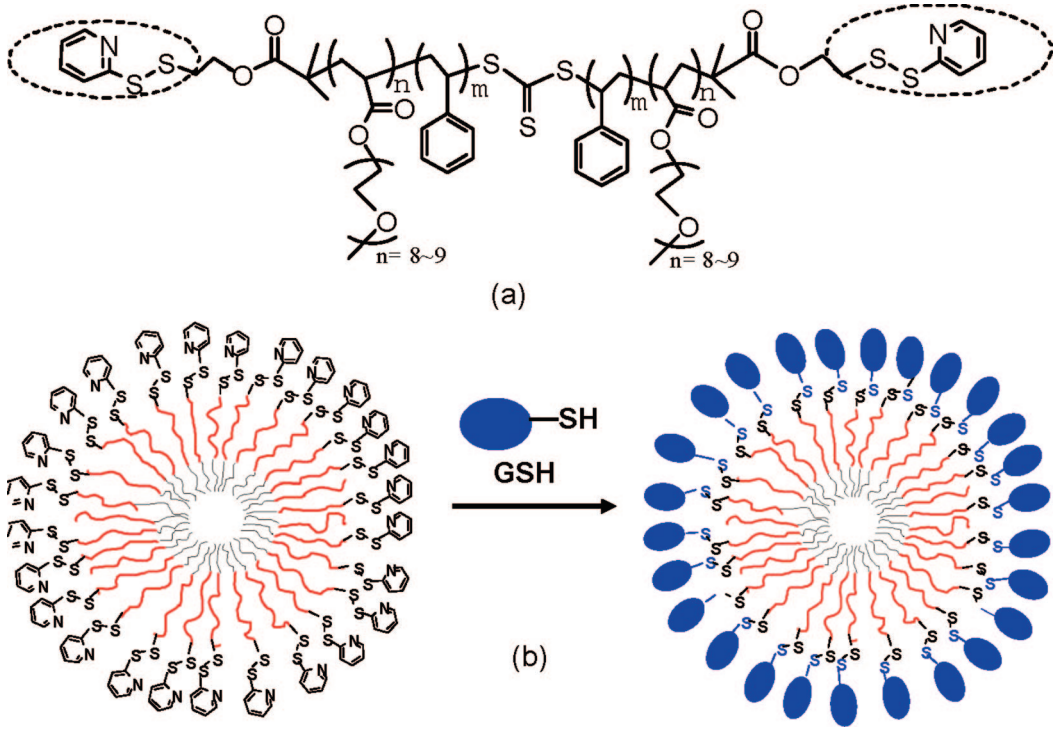
## 3.4. Folate Functionalized Assemblies

Combinations of living radical polymerization and azide−alkyne click chemistry have been employed to prepare temperature-responsive block copolymer micelles conjugated with biological ligands for active-cell targeting of therapeutic molecules.[200] Block copolymers of *N*-isopropylacrylamide (NIPAM) and *N*,*N*-dimethylacrylamide (DMA) were synthesized by RAFT polymerization with an azido chain transfer agent (CTA).[288] Cu(I)-catalyzed coupling with propargyl folate resulted in folate residues being efficiently conjugated to the R-azido chain ends of the homo and block copolymers. Temperature induced self-assembly resulted in aggregates capable of a controlled release of a model hydrophobic drug.

## 3.5. DNA/RNA Conjugates

Small interfering RNAs (siRNAs) are 21−23 base-paired oligonucleotides containing two nucleotide overhangs at 3′ ends. They have proven to be effective in silencing specific genes, conclusively demonstrating their potential as the next generation of therapeutic agents.[166] However, a bottleneck for efficient therapy via specific gene silencing lies in the inability to effectively deliver the siRNA. As a result,

**Scheme 15. A Diblock Copolymer with α- and ω-PDS Terminal Groups (a) and Its Micelles with Surface PDS Groups and the Subsequent Attachment of a Peptide, Glutathione (GSH)**




significant effort has been focused on developing methods to stabilize, increase circulation life times, and deliver siRNA to the cytoplasm of target tissues. Poly(ethylene glycol) (PEG) is the most common polymer used to protect siRNA.[289−291] In a recent work, siRNA was stabilized by the formation of reversible conjugates with poly(PEG acrylate) using RAFT polymerization.[144] It is reported that covalent attachment of PEG to siRNA or its delivery system can enhance stability and efficient delivery to targeted tissues.[144]

RAFT polymerization was employed to allow detector-free visualization of specific DNA sequences using dynamic polymer growth for signal amplification.[292] Chaix and co-workers reported the synthesis of oligonucleotide−polymer conjugates from the amphiphilic block copolymer of poly(*tert*-butylacrylamide-*block*-(*N*-acryloylmorpholine-*co*-*N*-acryloxysuccinimide)) using an original solid-phase DNA synthesis strategy. These oligonucleotide−block copolymer conjugates could be used as capture probes to amplify the responses of diagnostic assays *in vitro*.[188,189] Oligonucleotide−polymer conjugates were also synthesized via thiol−ene chemistry between ene-modified biomolecules and sulfhydryl terminated polymer generated from the aminolysis of RAFT polymer.[175]

## 3.6. Glycopolymers

Carbohydrates are well-known as fundamental building blocks and universal energy storage molecules in every living organism. They are now also known to play a key role in a plethora of biological processes involving cell−cell interaction, such as inflammation, viral infection, fertilization, and signal transmission. Glycomics, the study of the intricate carbohydrate biochemistry at work in these processes, is still in its infancy due to the high complexity of the glycocode resulting from the extremely high density of structural information of polysaccharides. Glycopolymers, synthetic macromolecules containing carbohydrate moieties, constitute a useful tool to decrypt the glycocode. Controlled radical polymerization, which enables the synthesis of well-defined polymers of a wide range of architectures and composition, is the best method to prepare glycopolymers.[293] The first glycopolymer synthesized by RAFT was reported in 2003.[294] 2-Methacryloxyethyl glucoside (2-MAOEGlc, **G1**) was polymerized directly in water at 70 °C in the presence of (4-cyanopentanoic acid)-4-dithiobenzoate. The polymerization was well controlled up to 40% conversion, after which molecular weight started to deviate from theory. Molecular weight distribution remained narrow throughout polymerization, but chain extension led to broader PDI. It is important to note that a small amount of sodium bicarbonate was needed to help the dissolution of the CTA. The same initiator/CTA system was studied by Albertin et al. in the polymerization of methyl 6-*O*-methacryloyl-D-glucoside (6-*O*-MMA-Glc, **G2**) in water.[295] Polymerization carried out in the presence of sodium carbonate or sodium bicarbonate showed an inhibition period of 60−90 min and $M_n$ values much higher than theory. These problems were circumvented by carrying out the polymerization in water/ethanol 90:10 mixtures. Poly(6-*O*-MMAGlc) and poly(2-MAOEGlc) obtained using this method could then be successfully chain extended with 2-hydroxyethyl methacrylate or methyl 6-*O*-methacryloyl-α-D-mannoside (6-*O*-MMA-Man, **G3**) to yield block copolymer of low PDI (1.20).[296,297] A detailed kinetic study of the RAFT polymerization of 6-*O*-MMA-Glc in water was later reported by Albertin and Cameron.[298] The chemoenzymatic procedure used to synthesize 6-*O*-MMA-Glc and 6-*O*-MMA-Man was also applied to afford 6-*O*-vinyladipoyl-D-glucopyranose (6-*O*-VA-Glu, **G4**). This monomer was polymerized in water in the presence of a dithiocarbamate CTA and in water/methanol with a xanthate-derivative. Narrow polydispersities were achieved.[296]

RAFT polymerization provides ease of access to block copolymers. Interesting hydrophilic−hydrophilic diblock glycopolymers have been synthesized via RAFT. Lowe and Wang[299] reported the synthesis of well-defined poly(3-*O*-methacryloyl-D-galactopyranose)-*block*-poly((2-dimethylamino)ethyl methacrylate) of various composition. The glycopolymer block was synthesized first from the protected 3-*O*-methacryloyl-1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (**G5**) in DMF at 60 °C using cumyl dithiobenzoate or cyanoisopropyl dithiobenzoate. Both CTA efficiently mediated the polymerization of the glycomonomer. DMAEMA was then polymerized in the presence of the glycopolymer macroCTAs. The PDI of the diblock copolymers remained below 1.20 in all the syntheses reported. The protected carbohydrate moieties were converted to free sugar block using TFA. This deprotection step did not affect the poly(DMAEMA) block. Narain's group prepared a range of double hydrophilic diblock copolymers containing a glycopolymer block.[300] Gluconolactone derivatives GAEMA (**G6**) and GAPMA (**G7**) were polymerized in a controlled manner in water/DMF mixtures using (4-cyanopentanoic acid)-4-dithiobenzoate as CTA. The macroCTAs obtained were used as precursors to synthesize polycationic second blocks of 2-aminoethyl methacrylamide hydrochloride (AEMA), 3-aminopropyl methacrylamide hydrochloride (APMA), or 2-methacryloyloxyethyl phosphorylcholine (MPC). The diblock copolymers' polydispersity remained below 1.40. Polycations are known for their ability to bind to DNA and for their toxicity toward living cells. Poly(APMA-*block*-GAPMA) was shown to form nanoparticles (sizes inferior to 100 nm) via complexation with plasmid DNA at physiological and slightly acidic pH. In addition, *in vitro* cytotoxicity studies were carried out on the HELA cell line. The glycopolymers were completely nontoxic; poly(GAPMA) even seemed to enhance cell proliferation. Poly(APMA) showed high toxicity over a range of concentration. Interestingly, poly(APMA-*block*-GAPMA) on the contrary was found to be biocompatible.

Amphiphilic block copolymers can self-assemble in solution to form micelles or vesicles.[120] This property is under intense scrutiny, as such self-assembled structures are strong candidates as nanoreactors, drug carriers, or even cell mimics. The usual procedure for synthesizing diblock copolymer via RAFT consists in the synthesis of a macroRAFT agent, which will then be used for the polymerization of the second monomer. Another way to utilize CuAAC was explored by Opsteen et al.[301] and applied by Ting and co-workers to the synthesis of poly(6-*O*-methacryloyl mannose-*block*-vinyl acetate).[302] Lectin binding experiments carried out with a homoglycopolymer of 6-*O*-methacryloyl mannose (**G8**) showed that modifying the 6-carbon position completely disrupted the protein−carbohydrate binding ability.[303] The conventional method was put to use by several groups to produce well-defined amphihiplic block copolymers containing one glycopolymer block and to examine their self-assembly in solution. Cameron et al. synthesized macroRAFT agents from 2-(-D-galactosyloxy)ethyl methacrylate (GalEMA, **G9**) and methyl 6-*O*-methacryloyl-α-D-glucoside (6-*O*-MMA-Glc, **G2**) in aqueous/ethanol solution and chain extended them with DMAEMA, BA, and BMA. A Poly(6-*O*-MMA-Glc-*block*-BMA) was seen to form aggregates and to encapsulate water-insoluble organic dye. Wormlike micelles were observed from the self-assembly of poly(GalEMA-*block*-BA).[304] Sanderson et al. prepared poly(3-*O*-methacryloyl glucopyranose-*block*-styrene) and poly(3-*O*-methacryloyl glucopyranose-*block*-methyl acrylate) by acidolysis of the corresponding isopropylidene-protected diblock copolymers (**G10**) obtained by RAFT. Critical micelle concentrations were determined to be around 0.12 g $L^{-1}$. Core−shell particles were observed from both water and toluene solution.[305] Thermoresponsive vesicles were synthesized by Pasparakis and Alexander[306] from the self-assembly of diblock copolymers of 2-glucosyloxyethyl methacrylate (2-MAOEGlc) and diethyleneglycol methacrylate (DEGMA) obtained by RAFT polymerization of the corresponding pentacetylated glycomonomer **G11**. Poly(2-MAOEGlc-*block*-DEGMA) spontaneously formed vesicles in water of mean diameter around 500 nm below 28 °C, the LCST of poly(DEGMA). Above that temperature, the vesicles shrank to around 300 nm with the collapse of the poly(DEGMA) block. These vesicles were shown to bind Concanavalin A better than linear poly(2-MAOEGlc). They could also bind a fluorescent mutant *E. coli* strain expressing receptor proteins specific to glucose and mannose. The authors showed that by choosing the size of the vesicles, which depends on the composition of the diblock copolymer, it was possible to obtain individual association between bacterium and vesicle, which could then be used to transfer the content of the vesicle to the bacterium. Oezyuerek et al. prepared thermoresponsive glycopolymers via RAFT polymerization from NIPAAm and a range of protected glycomonomers (**G10**, **G12**, and **G13**) varying in the length of the linker between the saccharide moiety and the polymerizable group.[307] Block copolymers were obtained from poly(NIPAAm) macroCTAs. The deprotection step was performed using formic acid after treatment with TFA proved to lead to ester bond cleavage. The LCST of the polymers was found to be strongly affected by both the structure of the copolymers (random or block) and the spacer length. Stenzel and co-workers further extended the study on thermosensitive glycopolymers via the use of an acid-degradable cross-linkers. Acryloyl glucosamine (AGA, **G14**) was used to synthesize a diblock copolymer with *N*-isopropylacrylamide which formed micelles at temperatures above the LCST of poly(NIPAAm). The polymers in these structures were then chain extended with an acetal-type cross-linking agent to afford core cross-linked micelles. These core−shell structures, stable at high pH, quickly decomposed to their unimers below pH 4.[308] Shell-cross-linked glycopolymer micelles were also prepared using a similar method. A polylactide macroRAFT agent was used to polymerize 1,2:3,4-di-*O*-isopropylidene-6-*O*-acryloyl-α-D-galactopyranose (**G15**). Acidolysis using formic acid afforded the amphiphilic diblock glycopolymer without degradation of the PLA block or of the trithiocarbonate moiety. Micelles were obtained in water, and these structures were stabilized by cross-linking of the shell using a diacrylate in a chain extension step. In a final step, aminolysis of the RAFT agent was performed to yield hollow particles whose shell is composed uniquely of poly(6-*O*-acryloyl-α-D-galactopyranose).[309] Stenzel's team further explored the use of RAFT to expand the architecture of glycopolymers. In particular, they examined the respective advantages and drawbacks of the R-group and Z-group approach for the synthesis of star glycopolymers. The superior R-group approach was put to use to prepare 4-arm star poly(6-*O*-VA-Glu) from a tetraxanthate derivative.[310] Using the Z-group approach, they prepared 3-arm star glycopolymers by sequentially polymerizing HEA and acryloyl glucosamine (AGA) in the presence of a trifunctional

RAFT agent. The short HEA block was necessary to obtain a water-soluble RAFT agent able to control the polymerization of AGA in a water−ethanol mixture. A loss of control was observed at high conversion.[311] The Z-group methodology was also employed to grow a thermoresponsive glycopolymer brush from silicon wafers. A block of poly(AGA) was first synthesized from a trithiocarbonate derivative immobilized on a silica surface through the Z-group. The glycopolymers were then chain extended with NIPAAm. Ellipsometry and contact angle measurements confirmed the controlled process and the structure of the brush.[312] Xiao et al. reported in 2008 the synthesis of an amphiphilic homoglycopolymer.[215] They used 1,2:3,4-di-*O*-isopropylidene-6-*O*-(2′-formyl-4′-vinylphenyl)-D-galactopyranose (IVDG, **G16**), a styrene derivative carrying a protected galactose moiety as well as an aldehyde group. The RAFT polymerization of IVDG in THF in the presence of 1-phenylethyl dithiobenzoate was well-controlled, and deprotection by acidolysis using formic acid yielded the glycopolymer. Micelles with a narrow size distribution decorated with galactose and aldehyde moieties were easily obtained and were used to bind bovine serum albumine through Schiff base linkage. Emulsion polymerization is an efficient process to produce functional particles. Bernard and co-workers used this technique to synthesize polysaccharide-coated submicrometeric particles. A dextran functionalized xanthate derivative synthesized via CuAAC was used to produce a stable latex of poly(vinyl acetate).[313] RAFT miniemulsion was also used to produce homopolymers of protected glycomonomers (**G10** and **G17**) as well as their block copolymers with BA and BMA.[314]

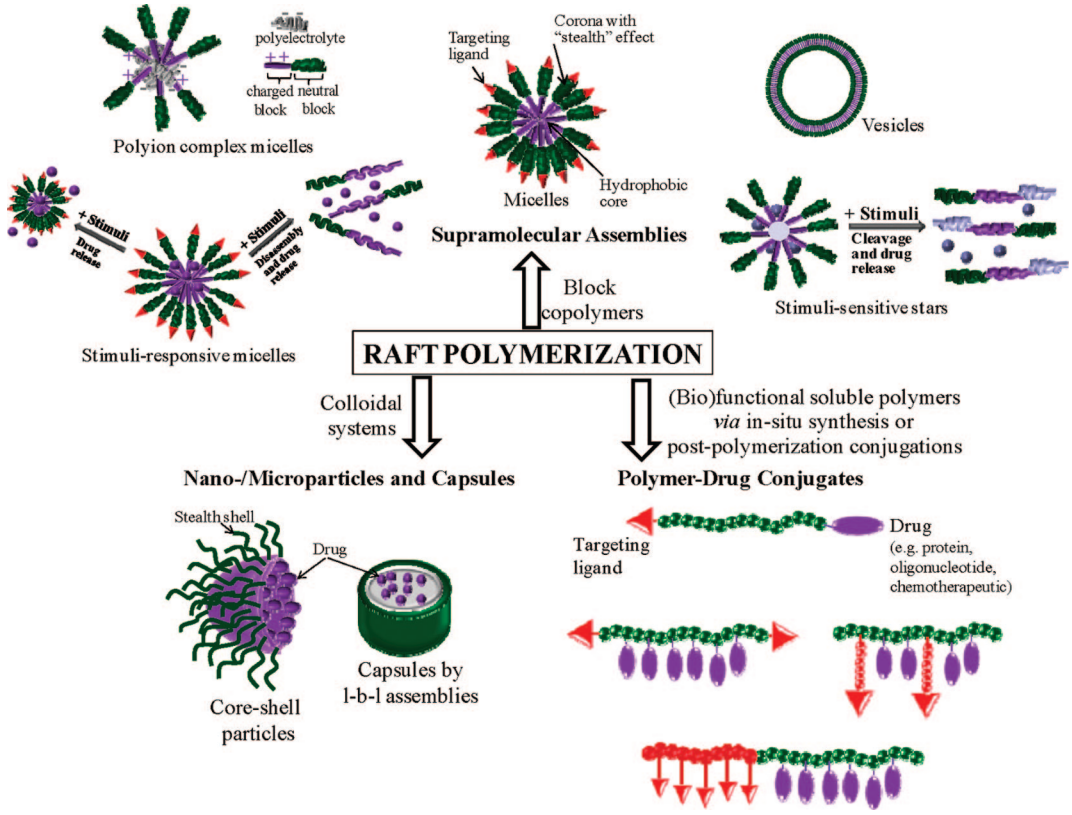
Confining a large number of sugar epitopes in a small volume is an efficient method to investigate the cluster glycoside effect: the phenomenon through which carbohydrates on a cell surface and lectins interact. This confinement can be done via self-assembly of amphiphilic polymers, as seen above or by attaching glycopolymers onto inorganic particles. Silica particles are an obvious substrate, for they present a reactive surface and are biocompatible and cheap. Guo and co-workers proposed an elegant way to graft silica particles with lactose containing polymers. Poly(2-*O*-methacryloyloxyethoxy-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1−4)-2,3,6-tri-*O*-acetyl-D-glucopyranoside) was synthesized via RAFT polymerization of the lactose monomer **G18** in chloroform using cumyl dithiobenzoate as CTA. This glycopolymer was then grafted onto γ-methacryloxypropyltrimethoxy-modified silica particles using AIBN as the source of radical. Deprotection using sodium methoxide yielded the particles grafted with well-defined lactose-containing polymer.[315] Gold nanoparticles (GNPs) constitute nanomaterials with unique properties in the fields of physicochemistry and biomedicine, thanks to their quantum-size effects.[316] They can be used as signal transducers, and saccharide modified GNPs have been used to monitor biological phenomena.[317−319] For these reasons, several groups have synthesized and studied the properties of glycopolymer-grafted gold nanoparticles. *p*-Acrylamidophenyl α-mannoside **G19** and *p*-acrylamidophenyl *N*-acetyl-β-glucosamine **G20** were homopolymerized and copolymerized with acrylamide in water/DMSO mixtures in the presence of (thiobenzoyl)thioglycolic acid. Thiol terminated glycopolymers were obtained by reduction of the dithiobenzoate moieties using $NaBH_4$. These reactive glycopolymers were then grafted to GNPs. Lectin binding assays were performed using concanavalin A and wheat germ agglutinin. The α-mannose-modified nanoparticles were also specifically recognized by a mutant strain of *E. coli*.[320] As shown by Spain et al., sodium borohydride can be used to simultaneously reduce dithiobenzoate terminated glycopolymers and $HAuCl_4$ to form glycopolymer-stabilized GNPs in a one-pot reaction.[321] They used this methodology to graft onto GNPs poly(2-(β-D-galactosyloxy)-ethylmethacrylate) obtained by RAFT from the unprotected galactose monomer **G21**. These particles strongly aggregated peanut agglutinin-coated agarose bead. The same strategy was used by Narain's group to graft biotinylated polyethylene glycol with poly(D-gluconamidoethyl methacrylate) (**G22**) or poly(2-lactobioamidoethyl methacrylate) (**G23**) onto GNPs. Aggregation of the particles upon addition of streptavidin as well as surface-plasmon resonance experiments proved the availability of biotin on the surface.[285] A similar strategy based on a photochemical process was also reported.[284] Biotinylated glycopolymers[283] from 6-*O*-acrylamido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**G24**) and poly(NIPAAm), both made by RAFT, were dissolved in an aqueous solution of $HauCl_4$ containing a thiol terminated polyethyleneglycol and Irgacure-2959, a photoinitiator. After UV irradiation, biotin decorated glyconoparticles were obtained. Biotin availability was investigated by SPR.

## 4. Drug Delivery

The historical evolution of controlled drug delivery systems (CDDS) from macro- to nanoscaled materials has been illustrated by Hoffman.[322] The emergence of the "polymer therapeutics" concept in the 1970s and the first clinical successes in 1980s and 1990s have revealed the potential of nanoscale tailored drug delivery systems for improving potent treatment strategies. In the last 20 years, an enormous effort in the drug delivery field has focused on generation of nanoscale constructs (i.e., nanomedicines), offering more efficient and safer ways for delivery of drugs. It is well-accepted that precise control over the hydrodynamic volume, morphology, chemical composition, and structure of polymers is necessary for generation of nanomedicines. With the ability to synthesize various architectures of a wide variety of polymers with defined end and pendant functionalities, controlled molecular weights, and narrow polydispersities using mild conditions (such as aqueous solutions and room temperatures), the RAFT technique appears to be one of the most amenable techniques to the generation of nanoscale polymeric systems for drug delivery.

The RAFT polymers have been increasingly used in potential drug delivery applications, as evidenced by the increasing number of publications in recent years. However, to the authors' knowledge, only one polymeric system generated by RAFT polymerization has been reported to be tested in *in vivo* experiments,[323] and only a few articles have reported the *in vitro* toxicity[110,324−326] and the blood-compatibility[327] data of varying RAFT-generated systems. It is well-known that the potential toxicity of thiocarbonylthio groups can be eliminated easily by postpolymerization treatments of the RAFT polymers.[63,328] However, toxicity assay results determined in a few published studies[110,324−326] (and also unpublished results of our group) suggest that the removal of the active thiocarbonylthio functionality from the RAFT-synthesized polymers may not always be necessary for *in vitro* experiments depending on the type of the RAFT agent (substituent groups), the type of the polymer and cells,

**Scheme 16. Examples of Controlled Drug Release Systems Generated by RAFT-Polymers: Stealth and/or Targeted Micelles and Vesicles, Stimuli-Responsive Micelles and Stars, Polyion Complexes, Polymer−Drug Conjugates, Particles, and Capsules**




and the concentration of the polymer used. While the systematic investigations on the pharmacological profile, such as metabolic cytotoxicity, of polymeric RAFT agents are yet to be performed, the general trend in the literature indicates that the RAFT polymerization would be used more commonly in the controlled drug delivery field in the near future as the technique starts to be used collaboratively by polymer chemists, material scientists, and biomedical researchers, and the RAFT agents become commonly available.

To date, efforts have focused on the use of RAFT polymerization for generating block copolymer micelles, vesicles, star polymers, nanoparticles, and capsules as potential advanced drug carriers and also polymer−drug conjugates as prodrugs (Scheme 16). The most commonly studied systems are reviewed in detailed below.

## 4.1. Supramolecular Assemblies: Micelles, Vesicles, and Stars

The self-assembly of amphiphilic di- and triblock copolymers into micelles[329,330] and vesicles (polymersomes)[331] has been investigated widely for developing CDDS potentially suitable for systemic administrations.[219,332] Therapeutic molecules can be incorporated into micelles and vesicles via hydrophobic interactions, electrostatic attractions, hydrogen, and/or covalent bonds. Biodistribution, stability, solubility, immunogenicity, and nonspecific bioactivity of therapeutics can be altered using micelles/vesicles rationally designed for a particular application. Micellar structures can be programmed to release the therapeutics upon an environmental-trigger such as temperature and pH or by passive diffusion, depending on the application.[333]

Immense attention in the RAFT polymerization field has been given to the generation of amphiphilic block copolymers as building blocks of micelles/vesicles for potential drug delivery applications. RAFT-generated amphiphilic copolymers have recently been reviewed in detail by others.[115,120] RAFT polymerization provides a versatile route to the generation of block copolymer micelles with controllable features, such as block lengths affecting the critical micelle concentration (thus stability), hydrodynamic size, and morphology, and chemical functionalities in the micelle corona and core offering possibilities to stabilize the supramolecular structure via covalent bonds (i.e., shell or core cross-linking), conjugating with biologically active molecules such as cell-specific targeting molecules and therapeutics.

### 4.1.1. Stealth Micelles/Vesicles

In general, the corona of micelles and vesicles should contain a suitable polymer, endowing a favorable "stealth" effect to the nanoassembly, minimizing the immunological reactions *in vivo* and prolonging blood-residence times. Amphiphilic block copolymers having a corona composed of an inert polymer having a stealth effect, such as PEG, poly(hydroxypropyl methacrylamide) (PHPMA), and a relatively less-common but promising candidate, poly(*N*-acryloylmorpholine) (PAM), have been generated recently by the RAFT polymerization for potential drug delivery applications.

In general, PEG and poly(ethylene oxide) (PEO) shielded micelles have been prepared by PEG- or PEO-based macroRAFT agents.[267,324] Li and co-workers have reported the reversible shell-cross-linking of PEO shielded micelles via disulfide bonds and the use of such micelles in controlled release of drugs such as a model bioactive agent, dipyridamole (DIP).[334] Cumulative DIP release from shell-cross-linked micelles exhibited a sustained release behavior compared to the micelles without shell-cross-linking. Zhu

et al. have reported the synthesis of micelles with a PEG corona and a cationic and hydrophobic double-layered core for simultaneous delivery of genes and hydrophobic drugs such as doxorubicin to increase the efficiency of chemotherapy in multidrug resistant cancer cells.[324] In this study, the amphiphilic block copolymers of PEG with poly(*N*-[3-(dimethylamino)propyl]methacrylamide-*co*-[2-hydroxyethylmethacryalte-poly(ε-caprolactone)]) were prepared by combining RAFT polymerization with ring-opening polymerization using a PEG-based macro RAFT agent. While the cationic block layer was used to complex with gene-based drug, the hydrophobic inner core was used to retain a hydrophobic anticancer drug, doxorubicin.

Using a different approach, Nystrom and co-workers have generated PEG-coated shell-cross-linked Knedel-like (SCK) block copolymer micelles harboring high loading of perfluorocarbons as potential magnetic resonance imaging (MRI) agents.[335] Poly(*tert*-butyl acrylate)-*block*-poly(styrene-*co*-2,3,4,5,6-pentafluorostyrene) was converted first to poly(acrylic acid)-*block*-poly(styrene-*co*-2,3,4,5,6-pentafluorostyrene) (PAAc-*block*-PS-co-PPFS) and then grafted with amine functionalized monomethoxy PEG.

It is also possible to replace PEG with its (meth)acrylate derivatives in preparation of stealth structures.[241] Accordingly, Zhang et al. have reported the RAFT-synthesis of shell-cross-linked micelles of poly(dimethylamino) ethyl methacrylate-*block*-poly(oligoethylene glycol) methacrylate[308] for delivery of genes. The toxicity of poly(dimethylamino) ethyl methacrylate to L929 fibroblasts was found to reduce significantly because of the shielding by the cross-linked poly(oligoethylene glycol) methacrylate corona despite the presence of thiocarbonylthio RAFT end groups on the polymer chains. In another study, RAFT-generated random terpolymers of oligoethylene glycol methacrylate, NIPAAm, and a cationic component, 3-(methylacryloylamino)propyl trimethylammonium chloride, were found not to alter the conformation of human serum albumin, suggesting the nonfouling effect of the oligoethylene glycol component.[212]

Poly(HPMA) is another well-known long-circulating, nonimmunogenic, hydrophilic polymer.[336] It has recently been synthesized via the RAFT polymerization, to yield polymers with controlled molecular weights and narrow polydispersities.[64] RAFT-synthesized well-defined amphiphilic block copolymers of poly(HPMA) have also been used to form micelles with a poly(HPMA) block forming hydrophilic corona, potentially improving the in vivo stability, nonimmunogenicity, and blood-circulation profiles of micelles.[104,132,166] Scales and co-workers have investigated a number of poly(HPMA-*block*-*N*-[3-(dimethylamino)propyl]methacrylamide) copolymers at varying compositions as potential vectors for small interfering RNAs (siRNA). Poly(HPMA) block stabilizes the interpolyelectrolyte complexes between the negatively charged siRNAs and the polycationic block, yielding nanosize complexes depending on the length of the poly(HPMA) block.[166] Bulmus and co-workers have generated well-defined amphiphilic block copolymers of HPMA with a thiol-reactive functional monomer[98] via the RAFT polymerization (Scheme 17).[104] The functional block enabled the conjugation of an antitumor drug, doxorubicin, to the polymer and concurrent cross-linking of the core via disulfide bonds cleavable in reducing environments, such as the cytoplasm of cells. Doxorubicin conjugated to the polymer via a hydrazine bond was released in its bioactive form by a low pH-trigger, making the system potentially suitable for intracellular[149] and tumor-site selective delivery of chemotherapeutics.

Poly(*N*-acryloylmorpholine) (PAM) is considered to have nonimmunogenic and long blood-circulation properties similar to those of PEG.[337−339] Jo and co-workers have recently synthesized homopolymers and block copolymers of *N*-acryloylmorpholine with *N*-acryloylpiperidine (AP) and *N*-acryloylazocane (AA) via the RAFT polymerization.[340] The formation of micelles or vesicles has been observed depending on the structure and composition of the block copolymers. A model hydrophobic drug, everolimus, exhibited a diffusion-driven, sustained release from PAM-*block*-PAH based micelles.

Polymers of phospholipids can improve biocompatibility and antithrombogenicity of drug delivery systems. A number of researchers have synthesized well-defined amphiphilic block copolymers of phosphoryl choline (MPC) and hydrophobic monomers such as *n*-butyl (meth)acrylate as potential biocompatible nanocarriers for hydrophobic drugs using the RAFT process.[326,341]

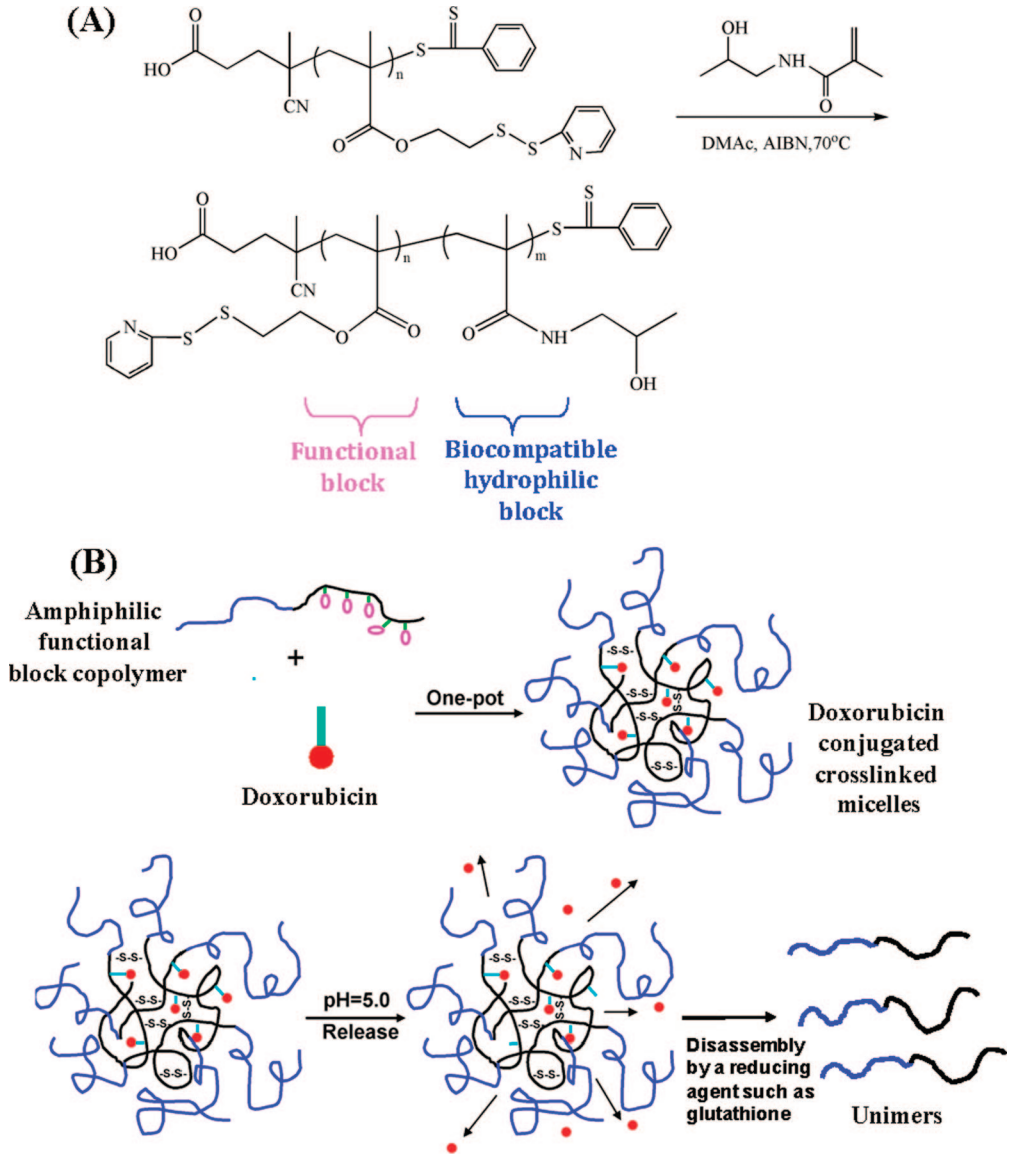
### 4.1.2. Stimuli-Responsive Micelles/Vesicles/Stars

Incorporation of stimuli-responsive behavior to the micelles and vesicles has been investigated widely to control the release of therapeutics and/or disassemble the supramolecular structure to unimers by environmental stimuli. Detailed reviews of stimuli-responsive nanocarriers including micelles and vesicles have been published elsewhere.[115,333,342−344] A wide variety of stimuli-sensitivity has been incorporated to RAFT-generated micelles/vesicles, stars, and branched structures for potential drug delivery applications (for example: pH-sensitive,[104,308,345−348] temperature-sensitive,[171,267,273,288,349−359] light-sensitive,[360] chemical and biological molecule sensitive)[104,211,334] A number of research groups[161,207,267,273,345,349,350,352] have reported temperature-responsive micelles composed of RAFT-generated copolymers of poly(NIPAAm), a well-known temperature-responsive polymer, as potential drug carriers. Differently, Fernandez-Trillo and co-workers have reported the RAFT-synthesis of well-defined elastin-based side-chain polymers (PDI 1.03−1.23) as temperature-responsive polymers having potential in varying biological applications including drug delivery.[359] Elastin-like peptides in aqueous solutions display reversible phase transitions as a result of temperature-dependent changes in the hydration state of the valine side chains.[361] Also, Lutz and his team have investigated the RAFT synthesis and temperature-responsive behavior of OEG-based polymers and their micellization.[118,362] Temperature-responsive OEG-based polymers are potentially of interest in drug delivery applications.

In addition to temperature, pH has also been investigated widely as an external stimulus for controlling the release from RAFT-generated micelles.[104,308,345−348] RAFT-synthesis of pH-responsive block copolymers of primary and tertiary amine containing monomers in aqueous media was performed by McCormick, Lowe, and co-workers.[345,346,355] It was possible to control the micellization and/or the hydrodynamic dimensions of the micelles by pH of the solutions and the composition of the polymers, which envisions the use of micelles for pH-controlled drug release. Such micelles were effectively stabilized via straightforward shell or core cross-linking strategies.

RAFT polymerization offers versatile cross-linking strategies for stabilization of the micelles. For example, it is

**Scheme 17. (A) Synthesis of Well-Defined Amphiphilic Block Copolymers of HPMA with a Thiol-Reactive Functional Monomer via the RAFT Polymerization and (B) Drug Conjugation, Micellization, and Core-Cross-linking in One-Pot, Followed by the Acid-Triggered Drug Release and Glutathione-Triggered Disassembly of Micelles[a]**




[a] Reprinted with permission from ref 104. Copyright 2008 American Chemical Society.

possible to cross-link the core of drug loaded micelles by chain extension polymerization from the living RAFT end groups of the core forming block with an acid-degradable bis-functional monomer at ambient temperatures to yield drug incorporated star polymers.[347] Such star polymers (or core cross-linked micelles) can be triggered by slight pH changes to disassemble to form unimers (under the cmc and above the cmc of the block copolymers) by the conversion of the hydrophobic core into a relatively more hydrophilic state, leading to the disassembly of micellar structure and the pH-controlled release of loaded drug (e.g., doxorubicin).[347] A wide variety of stimuli-sensitive cross-linked micellar systems and star polymers can be designed using the RAFT technique, as described in recent reviews.[115,120] The CAMD team have shown the synthesis of glutathione-sensitive cross-linked micelles[104] (Scheme 17) and star polymers,[102] which have potential for release of drugs in cell cytoplasm, where the concentration of glutathione, a natural tripeptide buffering the thiol-disulfide balance of cells,[363] can be as high as 10 mM. The synthesis of hyperbranched polymer has also been described for the encapsulation of drug or gene (siRNA).[102d−g,358] Recently, CAMD[102d−e] developed two different strategies for the synthesis of hyperbranched polymers built with disulfide bridges. The presence of disulfide bonds allows for a slow biodegradation of the hyperbranched structures. Dong et al.[102f] described the synthesis of hyperbranched polymers with pendent norbornene groups via the RAFT polymerization of a novel asymmetrical divinyl monomer bearing a higher reactivity methacrylate group ($r_1 = 12.5$) and a lower reactivity norbornene group ($r_2 = 0.078$). Rosselgong et al.[102h] proposed the synthesis of hyperbranched polymer using the copolymerization of MMA and a disulfide based dimethacrylate in the presence of cumyl dithiobenzoate. The disulfide bridges could be cleaved in the presence of tri-*n*-butyl phosphine.

Polyion complex micelles (PICs) are pH and/or ion-responsive micelle-like supramolecular nanostructures formed from a pair of oppositely charged block copolymers or a pair of oppositely charged block copolymer and homopolymer. They have potential in delivery of drugs, especially

charged therapeutics such as DNA, oligonucleotides, and proteins. A number of PICs have been prepared from the RAFT-generated copolymers composed of hydrophilic, neutral, and charged blocks, including the systems with dual mode responsiveness (e.g., temperature and pH-responsive) for potential drug delivery applications.[166,268,334,364−366] PICs formed from RAFT-generated poly(*N*-vinylpyrrolidone)-*block*-poly(styrene-alter-maleic anhydride) and poly(*N*-vinylpyrrolidone)-*block*-poly(*N*,*N*′-dimethylaminoethyl methacrylate) were used as controlled delivery vehicles for coenzyme A.[366] The release of biomolecules was 5-times faster at neutral pH compared to the release at acidic pH, suggesting potential as a colon-specific drug release system. RAFT-generated block copolymers of HPMA and the cationic monomer *N*-[3-(dimethylamino)propyl]methacrylamide (DMAPMA) were complexed with small interfering RNAs, yielding PHPMA stabilized PICs for potential gene silencing therapies.[166] siRNA within the complex exhibited enhanced resistance against nuclease degradation.

Bisht and co-workers[367] have prepared graft copolymers of poly(NIPAAm) with poly(ethyleneimine) (PEI) using RAFT polymerization and formed complexes with plasmid DNA, leading to the formation of polyion complex particles. They found that the cellular uptake and transfection activity of the DNA complexes with the PEI-g-poly(NIPAAm) copolymers was lower than those of the control PEI/DNA complexes at temperatures below the lower critical solution temperature (LCST) but increased to the PEI/DNA levels at temperatures above the LCST.[367] The same team used RAFT polymerization also to synthesize reducible poly(2-dimethylaminoethyl methacrylate) (rPoly(DMAEMA)) for fabrication of DNA complex particles. The rPoly(DMAEMA) polyplexes showed a comparable or better activity than control poly(DMAEMA) polyplexes.[368]

#### *4.1.3. Corona and Surface Functionalized Micelles/Vesicles*

Decoration of the outer surface of micelles with biological molecules is of great value for cell-specific targeting of drugs. RAFT polymerization enables the synthesis of alpha- and omega-functional and pendant-group functional block polymers suitable for conjugation with cell-specific targeting ligands to form cell-targeted micelles/vesicles.[147,215,288,306,368−370] Using the RAFT technique, it is possible to *in situ* generate biomolecule functionalized, amphiphilic block copolymers from biomolecule-modified RAFT agents[155] or biomolecule functionalized monomers.[306] Both approaches to generate biofunctionalized polymers have been reviewed in detail in the previous sections of this review. For example, De and co-workers have synthesized alpha-azido terminal temperature-responsive block copolymers from an azido-modified RAFT agent.[288] The azido end group of the copolymers was coupled efficiently with propargyl-modified folate (ligand for surface folate receptors overexpressed by certain cancer cells) via orthogonal click addition. While the exact morphology of the temperature-responsive nanoaggregates formed from folate functionalized block copolymers could not be determined, it is reasonable to assume that the block copolymer would form micelles in aqueous solution with a folate functionalized hydrophilic block exterior shell.

### 4.2. Particles/Capsules/Gels

The (nano)particles present a great interest for the drug delivery.[371] Before giving several examples of synthesis of particles by RAFT, it is important to define polymeric nanoparticles and microparticles in the context of drug delivery applications: nanoparticles are particles usually of 20−500 nm dimensions,[84] while microparticles have a size from 500 nm to several micrometers.

RAFT-generated well-defined polymers have also been investigated as building blocks of core−shell (nano/micro-)particles and capsules, generated by layer-by-layer (l-b-l) assemblies, for potential drug delivery applications.[164,372,373] Cortez and co-workers investigated the binding of core−shell type particles to LIM1215 cells, a colorectal cancer-derived cell line.[373] The particles, 1 $\mu$m in size, having a surface modified with RAFT-generated PEG-*block*-poly(4-styrenesulfonate) (PEG-*block*-PSS) and coated with humanized A33 antibodies (huA33 Ab) exhibited efficient cell-binding. The presence of PEG was found to enhance the specificity of receptor-mediated binding compared to the particles having a surface modified with only PSS and coated with huA33 Ab. Zelikin and co-workers have synthesized narrow polydisperse poly(*N*-vinylpyrrolidone) (PVP), a nonionic, nontoxic, and nonimmunogenic polymer, via RAFT polymerization.[164] After reduction of the thiocarbonlythio group, the generated free thiols were protected with Ellman's reagent for further reactions with fluorescent probes and biomolecules such as peptides and DNA. Adsorption of the polymer onto silica particles and the bioconjugations to the polymer layer on the particles were demonstrated, envisioning the use of the polymer in generation of l-b-l capsules modifiable with bioactive elements such as therapeutics and biomarkers.

RAFT polymerization has also been performed in dispersed media, which widens the synthetic possibilities for generating potential drug delivery systems. The RAFT technique in dispersed systems has recently been reviewed in detail by others.[38,266,374−376] To date, only limited work exists on investigation of nano- and microparticles generated via RAFT dispersed systems for potential drug delivery applications.[110,377] Chan et al. prepared acid-degradable, cross-linked core−shell particles (diameter $\approx$ 150−500 nm) composed of a poly(*n*-butyl acrylate) core and a poly(OEG-A) shell via the RAFT dispersion polymerization and investigated pH-controlled release of a model hydrophobic compound from the particles.[110]

Thermoreversible hdyrogels were also generated from RAFT-synthesized BAB triblock copolymers as potential tissue engineering scaffolds.[378] Well-defined poly(NIPAAm)-*block*-poly(*N*,*N*-dimethylacrylamide)-*block*-poly(NIPAAm) triblock copolymers underwent reversible physical gels above the phase transition temperature of poly(NIPAAm). The mechanical properties of the gels were found to be similar to those of collagen, a biopolymer used widely in tissue engineering applications. In addition, soluble polymers and gels of phosphate containing monomers were prepared by RAFT polymerization.[379] The calcification behavior of both polymers and gels in simulated body fluids was investigated for potential cell and tissue engineering applications. The amount of phosphate groups and the accessibility of the phosphates played an important role in both the amount and type of mineral formed.

### 4.3. Soluble Polymer−Drug Conjugates

Polymer conjugation to drugs can enhance the bioavailability of drugs by improving *in vivo* stability, biodistribution profile, solubility, and intracellular distribution of drugs.[79,84,86,87,219,322,380] It also enables the localization of a high concentration of drugs at a desired site of the body. Polymer−drug conjugates as prodrugs require the use of uniform polymers to be able to obtain a consistent *in vivo* profile with an identifiable structure−activity correlation. The RAFT technique offers an excellent platform for generation of well-defined, narrow polydisperse polymers with functional groups required for covalent conjugation of drugs and/or other functional elements.[144,196,323,327,381] Hence, it is suitable for preparation of drug−polymer conjugates and other soluble polymers designed to interact with biological systems for drug delivery applications.

Pan and co-workers have investigated the *in vivo* biodistribution and pharmacokinetic of radiolabeled poly(HPMA)−alendronate conjugates synthesized by RAFT polymerization.[323] A methacryloyl derivative of the bone-targeting agent alendronate was copolymerized with HPMA at 40 °C using a trithiocarbonate chain transfer agent and VA-044 as initiator. A number of copolymers with molecular weights ranging from 18 000 to 94 000 g/mol were obtained. In biodistribution experiments, the conjugates exhibited high binding affinity for bone. The accumulation of the conjugates in the liver and spleen depended on molecular weight and alendronate content. The half-life of the conjugates in blood circulation varied between 12.4 and 27.7 h, with the increase in the number average molecular weight being from 18 100 to 97 400 g/mol.

In another study, well-defined copolymers of OEG-MA with a protected aldehyde monomer were generated via RAFT polymerization using a trithiocarbonate chain transfer agent.[327] The aldehyde groups were conjugated with amine groups of an iron chelator, desferrioxamine (DFO), to develop a blood compatible and long-circulating macromolecular chelator which can bind iron in the body and be excreted through the kidney after degradation. Conjugation of DFO to the polymer led to an improvement greater than 100-fold in the cytotoxicity profiles against endothelial HUVEC cells. Furthermore, there was no indication that the polymer changed the coagulation properties of blood and caused the complement activation, suggesting its potential to stay in the vascular system without a major biological response for a long period of time.

Heredia and co-workers have prepared reversible conjugates of poly(OEG-A) with small interfering siRNAs to improve the serum stability.[144] α-Pyridyldisulfide terminated polymer was synthesized using a RAFT agent having a pyridyldisulfide modified R-group. A thiol modified siRNA was conjugated to the α-terminal of the polymer via disulfide bonds. The siRNA was able to release from the polymer under reducing conditions, suggesting the potential of the strategy for cytoplasmic release of siRNAs.

Hoffman and Stayton's team has reported membrane-disruptive polymers synthesized *via* the RAFT technique for intracellular drug delivery applications. pH-responsive well-defined poly(styrene-*alt*-maleic anhydride)alkylamide copolymers were synthesized for cytoplasmic delivery of proteins, peptides, and oligonucleotides.[381] The copolymers showed pH-dependent cell-membrane-destabilizing activity.[149,380] The activity was controlled by varying the length of the alkylamine groups, the degree of modification with the alkylamine, and the molecular weight of the copolymer. Separately, poly(*N*-isopropylacrylamide-*co*-propylacrylic acid) copolymers with narrow polydispersity were prepared using the RAFT process.[382] The copolymers showed pH and temperature tunable phase transition properties at the vicinity of physiologically relevant pH and temperature values.

## 5. Surface Modification by RAFT Polymerization for Biological Applications

Surface modification is an essential process in biotechnological applications such as tissue engineering, biosensors, or implants manufacturing. It is also widely used to regulate protein, microbial, and cell adhesion. Polymer coatings or the more recent grafting techniques are extremely useful to confer new properties to surfaces or various natures and shapes. In recent years, CRP techniques and RAFT in particular have been increasingly used to synthesize well-defined functional polymers for surface-modification. Here we present a literature review of the use of RAFT to modify surfaces for biotechnological applications. Two broad categories will be addressed: flat surfaces and particle surfaces.

### 5.1. Flat Surfaces

Perrier et al. reported one of the first examples of application of the RAFT process to modify a natural substrate: cellulose.[46] The technique developed consisted in the covalent binding of RAFT agents through their R-group to the hydroxyl groups of cellulose. The modified cellulose was then used in the surface-mediated RAFT polymerization of styrene.[383] A similar idea was developed by Barner and co-workers.[112] In that case, the styrene solution containing a dithiobenzoate and a piece of cellulose (filter paper) were irradiated with a $^{60}$Co source to induce the initiation site on the cellulose surface.[112] Both groups used styrene to modify the hydrophilicity of cellulose. Perrier further developed his process to the fabrication of the bioactive surface. Controlled poly(DMAEMA) chains were grown from a cellulose surface via RAFT and subsequently quaternized with alkyl bromides of various chain lengths. The surfaces quaternized with the shortest alkyl groups and of highest degree of quaternization exhibited high biocidal activity against *E. coli*.[384−386] Fleet et al.[387] reported the grafting of polymers by RAFT using the Z-group approach. Xanthate esters were formed directly onto hydroxypropyl cellulose and methyl cellulose. These modified substrates, allowing a higher density of grafting than cellulose, were used in the surface-mediated polymerization of vinyl acetate.[387] At high density of grafting, these materials could be considered as comb-shaped polymers with a backbone composed of a natural polymer and side chains of synthetic polymeric materials. Such a polymer was prepared by Hua and co-workers.[388] Chitosan was first transformed in *N*-phthaloylchitosan. RAFT agents were then attached to this DMF-soluble substance via esterification to provide anchoring points for the RAFT polymerization of acrylic acid. Peng et al. reported the preparation of a biocidal microfiltration membrane from a comb-shaped polymer prepared by RAFT.[389] Poly(vinylidene fluoride)-graft-poly(*N*-vinyl-2-pyrrolidone) was synthesized by RAFT polymerization of NVP with 1-phenylethyldithiobenzoate in the presence of an ozone-treated PVDF. Porous membranes were prepared by phase inversion in an aqueous medium from DMF solutions of the graft copolymer. The living PNVP chains on the surface of the membranes were chain extended

with DMAEMA by RAFT, and the poly(DMAEMA) block was quaternized with bromohexane. These membranes exhibited both antifouling and biocidal activity. The CAMD team[390−396] developed another interesting porous structure from RAFT polymers: the so-called honeycomb structured porous films. These porous structures, obtained from the self-assembly of condensed water droplets on top of a polymer solution, were prepared from a variety of comb-shaped polymers synthesized by RAFT, including a polystyrene comb grown from the hydroxypropyl cellulose backbone.[394] In addition, honeycomb porous films grafted to poly(NIPAAm) chains showed enhanced cell adhesion compared to that of the native PS-comb honeycomb film and to that of a nonporous poly(NIPAAm) grafted film.[396] Honeycomb porous films obtained from a polypyrrole containing poly((acrylic acid)-*block*-(styrene)) were shown to be noncytotoxic and suitable as a scaffold for cell growth.[395]

Surface-mediated RAFT polymerization was also examined to develop novel DNA-biosensors. Pirri et al. proposed to used RAFT to grow poly(dimethyl acrylamide-*block*-glycidyl methacrylate) diblock copolymers from glass slides and utilize these surface tethered polymers to immobilize DNA through reaction between DNA terminal amine and polyglycidyl block oxirane groups. Target DNA molecules are revealed by hybridization with a fluorescent DNA strand.[397] He and co-workers adapted to RAFT the DNA "amplification-by-polymerization" approach developed with ATRP.[398] Capture DNA immobilized on a surface can hybridize with a segment of the target DNA. A probe DNA complementary of the free segment of the target DNA carries a RAFT agent which can be used to grow a polymer brush. The polymer film thickness measured by ellipsometry is a direct evidence of the presence of the target DNA. This amplifying system enabled us to detect as few as 2000 copies of a short oligonucleotide.[292] Controlling cell adhesion on a surface is an important challenge that needs to be overcome in order to understand cell behavior and for applications in biomaterials or tissue engineering.[399,400] Maynard et al. reported an elegant way toward cell adhesion control that relies on the patterning of the growth factor on a surface: a poly(sodium 4-styrenesulfonate-*co*-poly(OEG-MA)) copolymer synthesized by RAFT in DMF/water mixtures. Poly(4-styrenesulfonate) can mimic the heparin onto which the target growth factors (vFGF and VEGF) bind very strongly, and poly(OEG-MA) can be cross-linked onto a silica surface by exposure to en electron beam. The polymer was spin-coated onto a silicon wafer from a methanol solution, and micro- and nanopatterns were created on the polymer film via electron-beam lithography. The protein adhesion on the polymer was detected by fluorescence microscopy.[401]

## 5.2. Particle Surfaces

Gold nanoparticles (GNPs, with size from 1 to 100 nm, but typically under 20 nm) have attracted increasing interest thanks to their optical properties, and they have been the substrate of choice to graft RAFT polymers, for their chain-end is easily directly reacted on a gold surface[402−404] or transformed into thiols.[246] We already mentioned a few examples of this method in the glycopolymer section.[283−285,320,321] Jerome et al. used this method to simultaneously prepare GNPs grafted with thiol terminated biotin functionalized poly(NIPAAm) obtained by RAFT.[126] As mentioned previously, silica particles have been used to graft lactose containing RAFT polymers.[315] Caruso et al. coated colloidal silica particles with thiol terminated poly(vinylpyrrolidone) (PVP) prepared by RAFT and use these free thiols for ligand immobilization. Fluorescent tag, short single strand oligonucleotides and oligopeptides were immobilized on the particle surface by disulfide bridges. Hybridization experiments were successfully conducted, and conditions for the desorption of the PVP from the silica particle surface were established.[164]

Gadolinium metal−organic framework (Gd MOF) nanoparticles (width of 20−25 nm and length of 100−150 nm) were modified by functional polymers obtained by RAFT polymerization to yield hybrid organic/inorganic nanoparticles. The synthesis of copolymers of poly(NIPAAm)-*co*-poly(NHS-A)-*co*-poly(fluorescein *O*-methacrylate) was achieved via RAFT polymerization. Using the succinimide group, the copolymers were decorated with a therapeutic agent, such as methotrexate, and a targeting ligand, such as H-glycine-arginine-glycine-aspartate-serine-$NH_2$ peptide. Finally, the reduction of the trithiocarbonate RAFT agent was accomplished to generate a thiol end group, providing a means of copolymer attachment through vacant orbitals on the $Gd^{3+}$ ions at the surface of the Gd MOF nanoparticles.[405] These particles can be used as an MRI agent and drug delivery system. The attachment of polymer on the surface of Gd MOF nanoparticles can affect positively (improve) the property of relaxivity of these particles.[406]

Semiconductor nanocrystals (usually called quantum dots) have a great interest for biomedical applications due to the high luminisence, single excitation, narrow emission, and low toxicity. Quantum dots can be exploited for in vivo labeling/imaging of cells. RAFT copolymerization of three different monomers containing amine, sugar, and biotin pendent groups was achieved. The polymer was attached on quantum dots using amine−carboxylic acid coupling. The polymer confers new properities (targeting) and improves the biocompatibility.[407]

Iron oxide nanoparticles (IONPs) (size inferior to 100 nm) are of great interest for researchers from a wide range of disciplines, including magnetic fluids, catalysis, biotechnology/biomedicine, magnetic resonance imaging, data storage, and environmental remediation.[408] When their dimensions fall below a certain value dependent on the materials but typically around 10−20 nm, they display superparamagnetic behavior. This behavior makes superparamagnetic NPs very attractive for a broad range of biomedical applications, because the risk of forming agglomerates is negligible at room temperature. So far, a narrow range of polymeric coatings has been used and RAFT has still been scarcely used for stabilization or further functionalization of IONPs. Narain et al. used a mixture of non-end-functionalized poly(NIPAAm) and biotinylated poly(NIPAAm) prepared by RAFT to stabilize IONPs and showed that the availability of the biotin on the particle surface could be turned on and off with temperature.[282] IONPs are considered in hyperthermia cancer therapy, but for this application to be successful, the nanoparticles need to form a stable colloidal suspension in physiological fluids and must not elicit an immune response. Jerome's team prepared and coated IONPS with a range of double hydrophilic diblock copolymers by RAFT. The diblock copolymers were composed of a block of poly(acrylic acid) and a block of linear polyethylene oxide or of poly(OEG-A). The grafting to method used led to IONP aggregates of 50−100 nm. The aggregates exhibited stealthi-

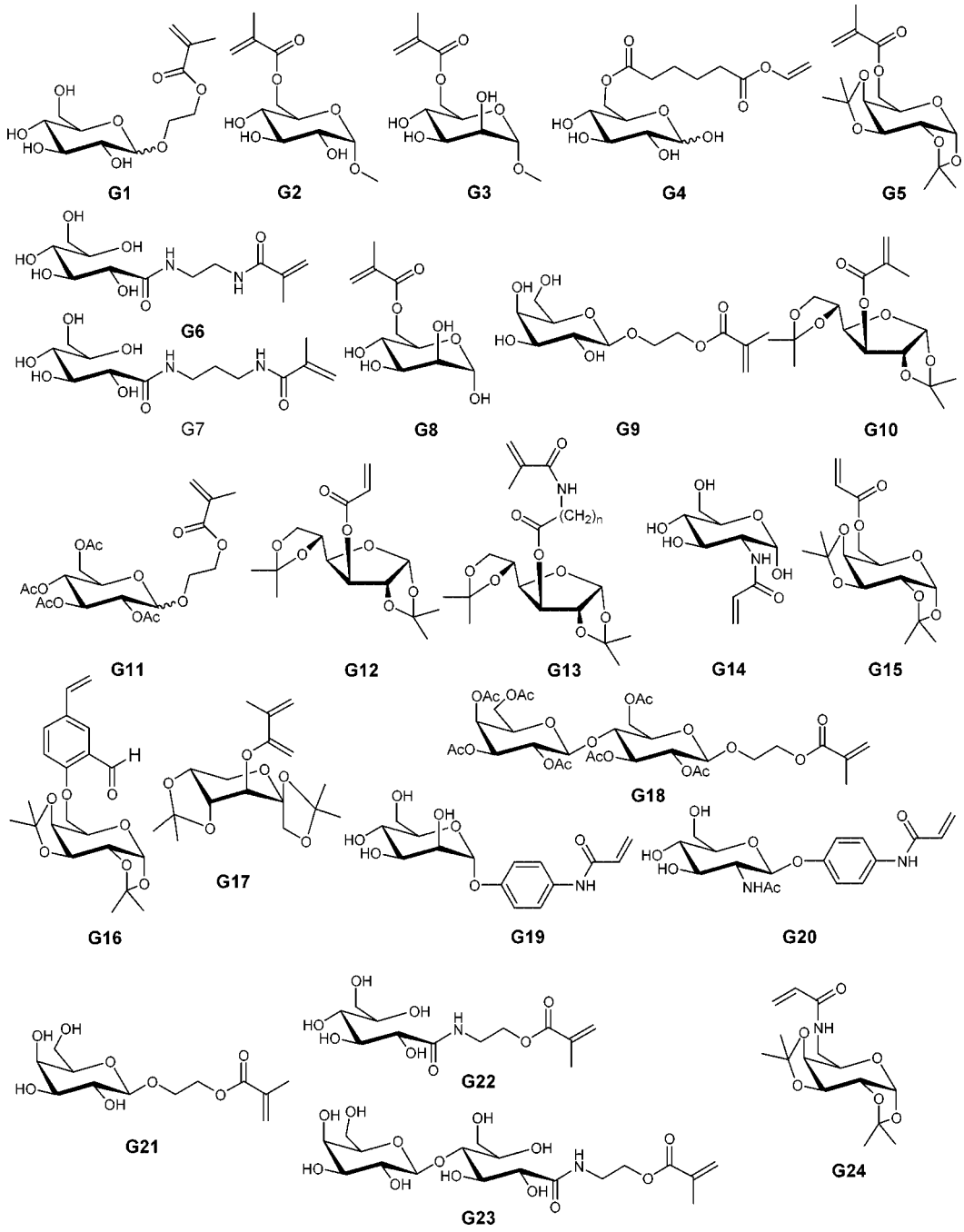



**Figure 6.** Glycomonomers polymerized by RAFT.

ness, as tested in vitro by the hemolytic CH50 test.[409,410] Tremel et al. reported a method to immobilize silicatein on IONPs and fabricate $Fe_2O_3$@$SiO_2$ core shell nanoparticles. A RAFT poly(pentafluorophenyl acrylate) was reacted with dopamine and an amino functionalized nitrilotriacetic acid (NTA) to yield a copolymer containing catecholate groups able to bind to IONPs and NTA groups. The coating of maghemite nanoparticles with this copolymer was reported to proceed without aggregation. Treatment of the polymer coated-IONPs with $NiSO_4$ and subsequent incubation with a recombinant silicatein containing a His-tag afforded the desired protein-decorated IONPs. The immobilized enzymes retained their activity, and shells of silica were formed around the IONPS via biomineralization.[411]

Boyer et al. reported an original method for attaching polypeptides to IONPs. A new trithiocarbonate RAFT agent bearing a dimethyl phosphonate group was synthesized and used to polymerize styrene, NIPAAm, and OEG-A. The dimethyl phosphonate α-end group was deprotected to yield a free phosphonic acid group. The trithiocarbonate chain-end was removed by aminolysis, and then, the free thiol obtained was in situ reacted with dithiopyridine. Telechelic poly(OEG-A) of molecular weight ranging from 6 000 to 62 000 g/mol was reacted with IONPS using the "grafting-to" approach. Grafting density as high as 0.2 chains $nm^{-2}$ was obtained. The particles grafted with 62 000 g/mol polymer were stable for 14 days in water and for 48 h in BSA-containing phosphate buffer. The particles also exhibited antifouling properties thanks to the poly(OEG-A) acting as a protein-repellent. Finally, the pyridyl disulfide group was used to decorate the polymer-stabilized particles with two peptides bearing free thiols: reduced glutathione and NGR motive.[93] The CAMD team[412] conjugated siRNA on the surface of IONPs, using the co-self-assembly of two

different polymers, i.e., poly(oligoethylene glycol) methyl ether acrylate (P(OEG-A)) and poly(dimethylaminoethyl acrylate) (P(DMAEA)). siRNA was complexed to the P(DMAEA) polymers, with the P(OEG-A) polymers imbuing the IONPs with anti-fouling and neutral surfaces. These hybrid organic/inorganic particles (70−150 nm) proved to be stable in both water and SD v% fetal bovine serum (FBS). Finally, these hybrid particles were evaluated for the transport and delivery of siRNA to human neuroblastoma SHEP cells.

## 6. Conclusion and Outlook

In this review we have restricted ourselves to the bioapplications of RAFT polymerization. The potential for designing novel polymeric structures via RAFT for bioapplications is clearly huge, and herein we have reviewed applications in biomaterials, drug delivery, gene therapy, glycopolymers, and bioconjugates. The field is rapidly expanding, and there are many more exciting and new opportunities to explore. One of the next big challenges is to transfer the systems presented herein to *in vivo* tests, including on humans. Indeed, due to the novelty of the field, *in vivo* studies, even in mice, are still rare. However, this next big step might not be as distant as it seems. One of the attractive features of RAFT polymerization is its simple setup, which makes it accessible to a vast number of research groups. As RAFT becomes established as a commonplace synthetic technique, it is likely to become a vector that favors interdisciplinary collaborations between polymer groups and research teams focused on bioapplications and within hospitals and medical research institutes. It is likely RAFT will allow these two research areas to work together and communicate with each other, to rapidly evolve and create products that would not have been possible without the collaborative effort and a practical technique in hand. There is no doubt in our mind that this review is of a nascent research field, and we hope it will help bring many more non-RAFT-specialists into the area.

## 7. Abbreviations

| | |
|---|---|
| AA | acrylic acid |
| AIBN | 2,2′-azobisisobutyronitrile |
| AM | acrylamide |
| AN | acrylonitrile |
| APDA | 3-acrylamidophenylboronic acid |
| ATRP | atom transfer radical polymerization |
| *n*-BA | *n*-butyl acrylate |
| *t*-BMA | *tert*-butyl methacrylate |
| *t*-Boc | *tert*-butyloxycarbonate |
| BSA | bovine serum albumin |
| CDB | cumyl dithiobenzoate |
| CDDS | controlled drug delivery systems |
| cmc | critical micelle concentration |
| CPAD | 4-cyanopentanoic acid dithiobenzoate |
| CTA | chain transfer agent |
| DEG MA | diethylene glycol monomethyl ether methacrylate |
| DEPMA | diethoxypropyl methacrylate |
| DMA | *N*,*N*-dimethylacrylamide |
| DMAEA | *N*,*N*′′-dimethylaminoethylacrylate |
| DMAEMA | *N*,*N*′′-dimethylaminoethylmethacrylate |
| DMAPMA | *N*-[3-(dimethylamino)propyl]methacrylamide |
| DMF | dimethylformamide |
| DTP | 2,2′-dithiopyridyl disulfide |
| DCM | dichloromethane |
| DCC | *N*,*N*′-dicyclohexylcarbodiimide |
| DMAP | 4-dimethyl aminopyridine |
| EDC | *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide |
| Et A | ethyl acrylate |
| FRP | free radical polymerization |
| GNPs | gold nanoparticles |
| HEA | 2-hydroxyethyl acrylate |
| HEMA | hydroxyethyl methacrylate |
| HPMA | hydroxyl propyl methacrylamide |
| IONPs | iron oxide nanoparticles |
| LCST | lower critical solution temperature |
| LMA | lauryl methacrylate (LMA) |
| LRP | living radical polymerization |
| MADIX | macromolecular design via the interchange of xanthates |
| MA | methyl acrylate |
| MMA | methyl methacrylate |
| NAM | *N*-acryloylmorpholine |
| NHS | *N*-succinimide |
| NIPAAm | *N*-isopropylacrylamide |
| NPMA | *p*-nitrophenyl methacrylate |
| NVP | *N*-vinylpyrrolidone |
| OEG | oligo(ethylene glycol) monomethyl ether |
| OEG A | oligo(ethylene glycol) monomethyl ether acrylate |
| OEG MA | oligo(ethylene glycol) monomethyl ether methacrylate |
| PAM | poly(*N*-acryloylmorpholine) |
| PDI | polydsipersity index |
| PDS | pyridyldisulfide |
| PEG | poly(ethylene glycol) |
| PEI | poly(ethyleneimine) |
| PEO | poly(ethylene oxide) |
| PSS | poly(4-styrenesulfonate) |
| PVDF | poly(vinylidene fluoride) |
| PVP | poly(*N*-vinylpyrrolidone) |
| poly(DMAEA) | poly(*N*,*N*′′-dimethylaminoethylacrylate) |
| poly(DMAEMA) | poly(*N*,*N*′′-dimethylaminoethylmethacrylate) |
| poly(HPMA) | poly(*N*-hydroxyl propyl methacrylamide) |
| poly(MMA) | poly(methyl methacrylate) |
| poly(NIPAAm) | poly(*N*-isopropylacrylamide) |
| RAFT | reversible addition−fragmentation chain transfer |
| siRNA | small interfering-RNAs |
| St | styrene |
| TFA | trifluoroacetic acid |
| VAc | binyl acetate |
| VBC | 4-vinylbenzoic acid |

## 8. References


(1) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661.
(2) Sciannamea, V.; Jerome, R.; Detrembleur, C. *Chem. Rev.* **2008**, *108*, 1104.
(3) Boyer, C.; Lacroix-Desmazes, P.; Robin, J.-J.; Boutevin, B. *Macromolecules* **2006**, *39*, 4044.
(4) David, G.; Boyer, C.; Tonnar, J.; Ameduri, B.; Lacroix-Desmazes, P.; Boutevin, B. *Chem. Rev.* **2006**, *106*, 3936.
(5) Percec, V.; Popov, A. V.; Ramirez-Castillo, E.; Hinojosa-Falcon, L. A. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2276.
(6) Percec, V.; Sienkowska, M. J. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 628.
(7) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559.
(8) Le, T. P.; Moad, G.; Rizzardo, E.; Thang, S. H. PCT Int. Appl. WO 9801478 A1 19980115, 1998.
(9) Charmot, D.; Corpart, P.; Adam, H.; Zard, S. Z.; Biadatti, T.; Bouhadir, G. *Macromol. Symp.* **2000**, *150*, 23.
(10) Corpart, P.; Charmot, D.; Biadatti, T.; Zard, S.; Michelet, D. PCT Int. Pat. Appl. WO 9858974 A1 19981230, 1998.
(11) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921.
(12) Tsarevsky, N. V.; Matyjaszewski, K. *Chem. Rev.* **2007**, *107*, 2270.
(13) Tang, H.; Arulsamy, N.; Radosz, M.; Shen, Y.; Tsarevsky, N. V.; Braunecker, W. A.; Tang, W.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2006**, *128*, 16277.

(14) Pintauer, T.; Matyjaszewski, K. *Chem. Soc. Rev.* **2008**, *37*, 1087.
(15) Percec, V.; Guliashvili, T.; Ladislaw, J. S.; Wistrand, A.; Stjerndahl, A.; Sienkowska, M. J.; Monteiro, M. J.; Sahoo, S. *J. Am. Chem. Soc.* **2006**, *128*, 14156.
(16) Sugihara, Y.; Kagawa, Y.; Yamago, S.; Okubo, M. *Macromolecules* **2007**, *40*, 9208.
(17) Yusa, S.-i.; Yamago, S.; Sugahara, M.; Morikawa, S.; Yamamoto, T.; Morishima, Y. *Macromolecules* **2007**, *40*, 5907.
(18) Yamago, S.; Iida, K.; Yoshida, J.-i. *J. Am. Chem. Soc.* **2002**, *124*, 13666.
(19) Kamigaito, M.; Ando, T.; Sawamoto, M. *Chem. Rev.* **2001**, *101*, 3689.
(20) Lu, L. C.; Yang, N. F.; Cai, Y. L. *Chem. Commun.* **2005**, 5287.
(21) Quinn, J. F.; Barner, L.; Barner-Kowollik, C.; Rizzardo, E.; Davis, T. P. *Macromolecules* **2002**, *35*, 7620.
(22) Barner, L.; Quinn, J. F.; Barner-Kowollik, C.; Vana, P.; Davis, T. P. *Eur. Polym. J.* **2003**, *39*, 449.
(23) Millard, P.-E.; Barner, L.; Stenzel, M. H.; Davis, T. P.; Barner-Kowollik, C.; Mueller, A. H. E. *Macromol. Rapid Commun.* **2006**, *27*, 821.
(24) Quinn, J. F.; Barner, L.; Rizzardo, E.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2001**, *40*, 19.
(25) You, Y.-Z.; Bai, R.-K.; Pan, C.-Y. *Macromol. Chem. Phys.* **2001**, *202*, 1980.
(26) Buback, M.; Junkers, T.; Vana, P. *Macromol. Rapid Commun.* **2005**, *26*, 796.
(27) Junkers, T.; Theis, A.; Buback, M.; Davis, T. P.; Stenzel, M. H.; Vana, P.; Barner-Kowollik, C. *Macromolecules* **2005**, *38*, 9497.
(28) McLeary, J. B.; Calitz, F. M.; McKenzie, J. M.; Tonge, M. P.; Sanderson, R. D.; Klumperman, B. *Macromolecules* **2005**, *38*, 3151.
(29) Chong, Y. K.; Krstina, J.; Le, T. P. T.; Moad, G.; Postma, A.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2003**, *36*, 2256.
(30) Moad, G.; Chiefari, J.; Chong, Y. K.; Krstina, J.; Mayadunne, R. T. A.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polym. Int.* **2000**, *49*, 993.
(31) Chiefari, J.; Mayadunne, R. T. A.; Moad, C. L.; Moad, G.; Rizzardo, E.; Postma, A.; Skidmore, M. A.; Thang, S. H. *Macromolecules* **2003**, *36*, 2273.
(32) Rizzardo, E.; Chiefari, J.; Mayadunne, R. T. A.; Moad, G.; Thang, S. H. *ACS Symp. Ser.* **2000**, *768*, 278.
(33) Stenzel, M. H.; Cummins, L.; Roberts, G. E.; Davis, T. P.; Vana, P.; Barner-Kowollik, C. *Macromol. Chem. Phys.* **2003**, *204*, 1160.
(34) Destarac, M.; Charmot, D.; Franck, X.; Zard, S. Z. *Macromol. Rapid Commun.* **2000**, *21*, 1035.
(35) Hua, D.; Xiao, J.; Bai, R.; Lu, W.; Pan, C. *Macromol. Chem. Phys.* **2004**, *205*, 1793.
(36) Mayadunne, R. T. A.; Rizzardo, E.; Chiefari, J.; Chong, Y. K.; Moad, G.; Thang, S. H. *Macromolecules* **1999**, *32*, 6977.
(37) Destarac, M.; Bzducha, W.; Taton, D.; Gauthier-Gillaizeau, I.; Zard, S. Z. *Macromol. Rapid Commun.* **2002**, *23*, 1049.
(38) Moad, G. *Aust. J. Chem.* **2006**, *59*, 661.
(39) Zhou, D.; Zhu, X.; Zhu, J.; Yin, H. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 4849.
(40) Perrier, S.; Barner-Kowollik, C.; Quinn, J. F.; Vana, P.; Davis, T. P. *Macromolecules* **2002**, *35*, 8300.
(41) Barner-Kowollik, C.; Buback, M.; Charleux, B.; Coote, M. L.; Drache, M.; Fukuda, T.; Goto, A.; Klumperman, B.; Lowe, A. B.; Mcleary, J. B.; Moad, G.; Monteiro, M. J.; Sanderson, R. D.; Tonge, M. P.; Vana, P. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 5809.
(42) Muthukrishnan, S.; Pan, E. H.; Stenzel, M. H.; Barner-Kowollik, C.; Davis, T. P.; Lewis, D.; Barner, L. *Macromolecules* **2007**, *40*, 2978.
(43) Aqil, A.; Detrembleur, C.; Gilbert, B.; Jerome, R.; Jerome, C. *Chem. Mater.* **2007**, *19*, 2150.
(44) Wang, W.-C.; Neoh, K.-G.; Kang, E.-T. *Macromol. Rapid Commun.* **2006**, *27*, 1665.
(45) Kulkarni, S.; Schilli, C.; Grin, B.; Mueller, A. H. E.; Hoffman, A. S.; Stayton, P. S. *Biomacromolecules* **2006**, *7*, 2736.
(46) Perrier, S.; Takolpuckdee, P.; Westwood, J.; Lewis, D. M. *Macromolecules* **2004**, *37*, 2709.
(47) Lima, V.; Jiang, X.; Brokken-Zijp, J.; Schoenmakers, P. J.; Klumperman, B.; Van Der Linde, R. *J. Polym. Sci., Part. A: Polym. Chem.* **2005**, *43*, 959.
(48) Benaglia, M.; Rizzardo, E.; Alberti, A.; Guerra, M. *Macromolecules* **2005**, *38*, 3129.
(49) Devasia, R.; Bindu, R. L.; Borsali, R.; Mougin, N.; Gnanou, Y. *Macromol. Symp.* **2005**, *229*, 8.
(50) (a) Nguyen, T. L. U.; Eagles, K.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4372. (b) Hussain, H.; Tan, B. H.; Gudipati, C. S.; Ye, L.; He, C. B.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 5604.
(51) Wan, D.; Satoh, K.; Kamigaito, M.; Okamoto, Y. *Macromolecules* **2005**, *38*, 10397.
(52) Shi, L.; Chapman, T. M.; Beckman, E. J. *Macromolecules* **2003**, *36*, 2563.
(53) Jitchum, V.; Perrier, S. *Macromolecules* **2007**, *40*, 1408.
(54) Convertine, A. J.; Sumerlin, B. S.; Thomas, D. B.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 4679.
(55) Tang, C.; Kowalewski, T.; Matyjaszewski, K. *Macromolecules* **2003**, *36*, 8587.
(56) Venkatesh, R.; Staal, B. B. P.; Klumperman, B. *Chem. Commun.* **2004**, 1554.
(57) Chen, M.; Ghiggino, K. P.; Smith, T. A.; Thang, S. H.; Wilson, G. J. *Aust. J. Chem.* **2004**, *57*, 1175.
(58) Gemici, H.; Legge, T. M.; Whittaker, M.; Monteiro, M. J.; Perrier, S. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 2334.
(59) Kakwere, H.; Perrier, S. *J. Am. Chem. Soc.* **2009**, *131*, 1889.
(60) Legge, T. M.; Slark, A. T.; Perrier, S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 6980.
(61) Moad, G.; Chong, Y. K.; Postma, A.; Rizzardo, E.; Thang, S. H. *Polymer* **2005**, *46*, 8458.
(62) Patton, D. L.; Mullings, M.; Fulghum, T.; Advincula, R. C. *Macromolecules* **2005**, *38*, 8597.
(63) Perrier, S.; Takolpuckdee, P.; Mars, C. A. *Macromolecules* **2005**, *38*, 2033.
(64) Scales, C. W.; Vasilieva, Y. A.; Convertine, A. J.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2005**, *6*, 1846.
(65) Segui, F.; Qiu, X. P.; Winnik, F. M. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 314.
(66) Spruell, J. M.; Levy, B. A.; Sutherland, A.; Dichtel, W. R.; Cheng, J. Y.; Stoddart, J. F.; Nelson, A. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *46*, 346.
(67) Whittaker, M. R.; Goh, Y. K.; Gemici, H.; Legge, T. M.; Perrier, S.; Monteiro, M. J. *Macromolecules* **2006**, *39*, 9028.
(68) Zhou, N.; Lu, L.; Zhu, X.; Yang, X.; Wang, X.; Zhu, J.; Zhou, D. *Polym. Bull.* **2006**, *57*, 491.
(69) Chong, B.; Moad, G.; Rizzardo, E.; Skidmore, M.; Thang, S. H. *Aust. J. Chem.* **2006**, *59*, 755.
(70) Tsujii, Y.; Ejaz, M.; Sato, K.; Goto, A.; Fukuda, T. *Macromolecules* **2001**, *34*, 8872.
(71) Patton, D. L.; Advincula, R. C. *Macromolecules* **2006**, *39*, 8674.
(72) Carter, S.; Hunt, B.; Rimmer, S. *Macromolecules* **2005**, *38*, 4595.
(73) Vogt, A. P.; Gondi, S. R.; Sumerlin, B. S. *Aust. J. Chem.* **2007**, *60*, 396.
(74) Liu, B.; Kazlauciunas, A.; Guthrie, J. T.; Perrier, S. *Macromolecules* **2005**, *38*, 2131.
(75) Vo, C. D.; Rosselgong, J.; Armes, S. P.; Billingham, N. C. *Macromolecules* **2007**, *40*, 7119.
(76) Barner-Kowollik, C.; Davis, T. P.; Stenzel, M. H. *Aust. J. Chem.* **2006**, *59*, 719.
(77) Mayadunne, R. T. A.; Jeffery, J.; Moad, G.; Rizzardo, E. *Macromolecules* **2003**, *36*, 1505.
(78) Boschmann, D.; Vana, P. *Macromolecules* **2007**, *40*, 2683.
(79) Ringsdorf, H. *J. Polym. Sci. Symp.* **1975**, *51*, 135.
(80) Ratner, B. D. *J. Biomed. Mater. Res.* **1993**, *27*, 837.
(81) Davis, F. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 457.
(82) Becker, M. L.; Liu, J.; Wooley, K. L. *Chem. Commun.* **2003**, 180.
(83) Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *46*, 6387.
(84) Duncan, R. *Nat. Rev. Cancer* **2006**, *6*, 688.
(85) Mitragotri, S.; Lahann, J. *Nat. Mater.* **2009**, *8*, 15.
(86) Pack, D. W.; Hoffman, A. S.; Pun, S.; Stayton, P. S. *Nat. Rev. Drug Discovery* **2005**, *4*, 581.
(87) Bartulin, J.; Przybyls, M.; Ringsdorf, H.; Ritter, H. *Makromol. Chem.* **1974**, *175*, 1007.
(88) Monzur Ali, S. B. *Adv. Drug Delivery Rev.* **2006**, *58*, 1671.
(89) Shaunak, S.; Godwin, A.; Choi, J.-W.; Balan, S.; Pedone, E.; Vijayarangam, D.; Heidelberger, S.; Teo, I.; Zloh, M.; Brocchini, S. *Nat. Chem. Biol.* **2006**, *2*, 312.
(90) Convertine, A. J.; Lokitz, B. S.; Vasileva, Y.; Myrick, L. J.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2006**, *39*, 1724.
(91) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379.
(92) McCormick, C. L.; Lowe, A. B. *Acc. Chem. Res.* **2004**, *37*, 312.
(93) Boyer, C.; Bulmus, V.; Priyanto, P.; Teoh, W. Y.; Amal, R.; Davis, T. P. *J. Mater. Chem.* **2009**, *19*, 111.
(94) Boyer, C.; Liu, J.; Bulmus, V.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromolecules* **2008**, *41*, 5641.
(95) Liu, J.; Bulmus, V.; Barner-Kowollik, C.; Stenzel, M. H.; Davis, T. P. *Macromol. Rapid Commun.* **2007**, *28*, 305.
(96) Postma, A.; Davis, T. P.; Evans, R. A.; Li, G.; Moad, G.; O'Shea, M. S. *Macromolecules* **2006**, *39*, 5293.
(97) Scales, C. W.; Convertine, A. J.; McCormick, C. L. *Biomacromolecules* **2006**, *7*, 1389.
(98) Wong, L.; Boyer, C.; Jia, Z.; Zareie, H. M.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 1934.
(99) York, A. W.; Scales, C. W.; Huang, F.; McCormick, C. L. *Biomacromolecules* **2007**, *8*, 2337.

(100) Stenzel, M. H.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 4498.
(101) Quinn, J. F.; Chaplin, R. P.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 2956.
(102) (a) Liu, J. Q.; Liu, H. Y.; Jia, Z. F.; Bulmus, V.; Davis, T. P. *Chem. Commun.* **2008**, 6582. (b) Liu, J.; Tao, L.; Xu, J.; Jia, Z.; Boyer, C.; Davis, T. P. *Polymer* **2009**, *50* (19), 4455. (c) Setijadi, E.; Tao, L.; Liu, L.; Jia, Z.; Boyer, C.; Davis, T. P. *Biomacromolecules* **2009**, *10*, 2699. (d) Xu, J.; Tao, L.; Liu, J.; Bulmus, V.; Davis, T. P. *Macromolecules* **2009**, *42*, 6893. (e) Tao, L.; Liu, J.; Tan, B. H.; Davis, T. P. *Macromolecules* **2009**, *42*, 4960. (f) Dong, Z.-M.; Liu, X.-H.; Tang, X.-I.; Li, Y. S. *Macromolecules* **2009**, *42* (13), 4596. (g) Dong, Z.-M.; Liu, X.-H.; Li, Y. S. *Chin. J. Polym. Sci.* **2009**, *27* (2), 285. (h) Rosselgong, J.; Armes, S. A.; Barton, W.; Price, A. *Macromolecules* **2009**, *42* (16), 5919.
(103) Boutevin, B.; David, G.; Boyer, C. *Adv. Polym. Sci.* **2007**, *206*, 31.
(104) Jia, Z. F.; Wong, L. J.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 3106.
(105) Boutevin, B.; Boyer, C.; David, G.; Lutz, P. In *Macromolecular Engineering*; Matyjaszewski, K. G., Yves, Leibler, L., Eds.; Wiley-VCH Verlag GmbH & Co.: Weinheim, 2007; Vol. 2.
(106) Biasutti, J. D.; Davis, T. P.; Lucien, F. P.; Heuts, J. P. A. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2001.
(107) Simms, R. W.; Davis, T. P.; Cunningham, M. F. *Macromol. Rapid Commun.* **2005**, *26*, 592.
(108) Lansalot, M.; Davis, T. P.; Heuts, J. P. A. *Macromolecules* **2002**, *35*, 7582.
(109) Barner, L.; Li, C. e.; Hao, X.; Stenzel, M. H.; Barner-Kowollik, C.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5067.
(110) Chan, Y.; Bulmus, V.; Zareie, M. H.; Byrne, F. L.; Barner, L.; Kavallaris, M. *J. Controlled Release* **2006**, *115*, 197.
(111) Barsbay, M.; Gueven, O.; Davis, T. P.; Barner-Kowollik, C.; Barner, L. *Polymer* **2009**, *50*, 973.
(112) Barsbay, M.; Gueven, O.; Stenzel, M. H.; Davis, T. P.; Barner-Kowollik, C.; Barner, L. *Macromolecules* **2007**, *40*, 7140.
(113) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *J. Am. Chem. Soc.* **2007**, *129*, 7145.
(114) Liu, J.; Bulmus, V.; Herlambang, D. L.; Barner-Kowollik, C.; Stenzel, M. H.; Davis, T. P. *Angew. Chem., Int. Ed.* **2007**, *46*, 3099.
(115) York, A. W.; Kirkland, S. E.; McCormick, C. L. *Adv. Drug Delivery Rev.* **2008**, *60*, 1018.
(116) Lowe, A. B.; McCormick, C. L. *Prog. Polym. Sci.* **2007**, *32*, 283.
(117) Nicolas, J.; Mantovani, G.; Haddleton, D. M. *Macromol. Rapid Commun.* **2007**, *28*, 1083.
(118) Lutz, J. F. *Polym. Int.* **2006**, *55*, 979.
(119) Gauthier, M. A.; Gibson, M. I.; Klok, H. A. *Angew. Chem., Int. Ed. Engl.* **2009**, *48*, 48.
(120) Stenzel, M. H. *Chem. Commun.* **2008**, 3486.
(121) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754.
(122) Wang, R.; McCormick, C. L.; Lowe, A. B. *Macromolecules* **2005**, *38*, 9518.
(123) Sumerlin, B. S.; Donovan, M. S.; Mitsukami, Y.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2001**, *34*, 6561.
(124) Vasilieva, Y. A.; Thomas, D. B.; Scales, C. W.; McCormick, C. L. *Macromolecules* **2004**, *37*, 2728.
(125) Donovan, M. S.; Sanford, T. A.; Lowe, A. B.; Sumerlin, B. S.; Mitsukami, Y.; McCormick, C. L. *Macromolecules* **2002**, *35*, 4570.
(126) Aqil, A.; Qiu, H. J.; Greisch, J. F.; Jerome, R.; De Pauw, E.; Jerome, C. *Polymer* **2008**, *49*, 1145.
(127) Bathfield, M.; D'Agosto, F.; Spitz, R.; Charreyre, M.-T.; Delair, T. *J. Am. Chem. Soc.* **2006**, *128*, 2546.
(128) Bathfield, M.; Daviot, D.; D'Agosto, F.; Spitz, R.; Ladaviere, C.; Charreyre, M.-T.; Delair, T. *Macromolecules* **2008**, *41*, 8346.
(129) Xu, J.; Boyer, C.; Bulmus, V.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47* (17), 4302.
(130) Aamer, K. A.; Tew, G. N. *J. Polym. Sci., Part. A: Polym. Chem.* **2007**, *45*, 5618.
(131) Roth, P. J.; Wiss, K. T.; Zentel, R.; Theato, P. *Macromolecules* **2008**, *41*, 8513.
(132) Barz, M.; Tarantola, M.; Fischer, K.; Schmidt, M.; Luxenhofer, R.; Janshoff, A.; Theato, P.; Zentel, R. *Biomacromolecules* **2008**, *9*, 3114.
(133) Wiss, K. T.; Krishna, O. D.; Roth, P. J.; Kiick, K. L.; Theato, P. *Macromolecules* **2009**, *42*, 3860.
(134) Vora, A.; Nasrullah, M. J.; Webster, D. C. *Macromolecules* **2007**, *40*, 8586.
(135) Zhang, L.; Chen, Y. *Polymer* **2006**, *47*, 5259.
(136) Campos, L. M.; Killops, K. L.; Sakai, R.; Paulusse, J. M. J.; Damiron, D.; Drockenmuller, E.; Messmore, B. W.; Hawker, C. J. *Macromolecules* **2008**, *41*, 7063.
(137) Strandwitz, N. C.; Khan, A.; Boettcher, S. W.; Mikhailovsky, A. A.; Hawker, C. J.; Nguyen, T.-Q.; Stucky, G. D. *J. Am. Chem. Soc.* **2008**, *130*, 8280.
(138) Karunakaran, R.; Kennedy, J. P. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4254.
(139) Bays, E.; Tao, L.; Chang, C.-W.; Maynard, H. D. *Biomacromolecules* **2009**, *10*, 1777.
(140) Zhou, N.; Lu, L.; Zhu, J.; Yang, X.; Wang, X.; Zhu, X.; Zhang, Z. *Polymer* **2007**, *48*, 1255.
(141) Boyer, C.; Otazaghine, B.; Boutevin, B.; Joly-Duhamel, C.; Robin, J.-J. *J. Polym. Sci., Part. A: Polym. Chem.* **2005**, *43*, 4303.
(142) Zugates, G. T.; Anderson, D. G.; Little, S. R.; Lawhorn, I. E. B.; Langer, R. *J. Am. Chem. Soc.* **2006**, *128*, 12726.
(143) Boyer, C.; Liu, J.; Wong, L.; Tippett, M.; Bulmus, V.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7207.
(144) Heredia, K. L.; Nguyen, T. H.; Chang, C. W.; Bulmus, V.; Davis, T. P.; Maynard, H. D. *Chem. Commun.* **2008**, 3245.
(145) Zareie, H. M.; Boyer, C.; Bulmus, V.; Nateghi, E.; Davis, T. P. *ACS Nano* **2008**, *2*, 757.
(146) Murthy, N.; Campbell, J.; Fausto, N.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **2003**, *14*, 412.
(147) Liu, J.; Liu, H.; Bulmus, V.; Boyer, C.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 899.
(148) Heredia, K. L.; Grover, G. N.; Tao, L.; Maynard, H. D. *Macromolecules* **2009**, *42*, 2360.
(149) Bulmus, V. *Aust. J. Chem.* **2005**, *58*, 411.
(150) Le Droumaguet, B.; Velonia, K. *Macromol. Rapid Commun.* **2008**, *29*, 1073.
(151) Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *Macromol. Rapid Commun.* **2008**, *29*, 1172.
(152) Chen, F.; Cheng, Z.; Zhu, J.; Zhang, W.; Zhu, X. *Eur. Polym. J.* **2008**, *44*, 1789.
(153) Ladmiral, V.; Legge, T. M.; Zhao, Y.; Perrier, S. *Macromolecules* **2008**, *41*, 6728.
(154) Favier, A.; Barner-Kowollik, C.; Davis, T. P.; Stenzel, M. H. *Macromol. Chem. Phys.* **2004**, *205*, 925.
(155) Hong, C.-Y.; Pan, C.-Y. *Macromolecules* **2006**, *39*, 3517.
(156) Hentschel, J.; ten Cate, M. G. J.; Borner, H. G. *Macromolecules* **2007**, *40*, 9224.
(157) Ten Cate, M. G. J.; Boerner, H. G. *ACS Symp. Ser.* **2006**, *944*, 198.
(158) Ten Cate, M. G. J.; Boerner, H. G. *Macromol. Chem. Phys.* **2007**, *208*, 1437.
(159) Ten Cate, M. G. J.; Rettig, H.; Bernhardt, K.; Boerner, H. G. *Macromolecules* **2005**, *38*, 10643.
(160) Zhao, Y.; Perrier, S. *Chem. Commun.* **2007**, 4294.
(161) De, P.; Li, M.; Gondi, S. R.; Sumerlin, B. S. *J. Am. Chem. Soc.* **2008**, *130*, 11288.
(162) Chang, C.-W.; Bays, E.; Tao, L.; Alconcel, S. N. S.; Maynard, H. D. *Chem. Commun.* **2009**, 3580.
(163) Pound, G.; McKenzie, J. M.; Lange, R. F. M.; Klumperman, B. *Chem. Commun.* **2008**, 3193.
(164) Zelikin, A. N.; Such, G. K.; Postma, A.; Caruso, F. *Biomacromolecules* **2007**, *8*, 2950.
(165) Qiu, X.-P.; Winnik, F. M. *Macromol. Rapid Commun.* **2006**, *27*, 1648.
(166) Scales, C. W.; Huang, F. Q.; Li, N.; Vasilieva, Y. A.; Ray, J.; Convertine, A. J.; McCormick, C. L. *Macromolecules* **2006**, *39*, 6871.
(167) Segui, F.; Qiu, X.-P.; Winnik, F. M. *J. Polym. Sci., Part. A: Polym. Chem.* **2007**, *46*, 314.
(168) Qiu, X.-P.; Tanaka, F.; Winnik, F. M. *Macromolecules* **2007**, *40*, 7069.
(169) Li, M.; De, P.; Gondi, S. R.; Sumerlin, B. S. *J. Polym. Sci., Part. A: Polym. Chem.* **2008**, *46*, 5093.
(170) Xu, J.; He, J.; Fan, D.; Wang, X.; Yang, Y. *Macromolecules* **2006**, *39*, 8616.
(171) Akimoto, J.; Nakayama, M.; Sakai, K.; Okano, T. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7127.
(172) You, Y. Z.; Oupicky, D. *Biomacromolecules* **2007**, *8*, 98.
(173) An, Z.; Tang, W.; Wu, M.; Jiao, Z.; Stucky, G. D. *Chem. Commun.* **2008**, 6501.
(174) (a) Yu, B.; Chan, J. W.; Hoyle, C. E.; Lowe, A. B. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 3544. (b) Li, H.; Yu, B.; Matsushima, H.; Hoyle, C. E.; Lowe, A. B. *Macromolecules* **2009**, *42* (17), 6537.
(175) Boyer, C.; Bulmus, V.; Davis, T. P. *Macromol. Rapid Commun.* **2009**, *30*, 493.
(176) Boyer, C.; Liu, J.; Bulmus, V.; Davis, T. P. *Aust. J. Chem.* **2009**, *62* (8), 830.
(177) Boyer, C.; Granville, A.; Davis, T. P.; Bulmus, V. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47* (15), 3773.
(178) (a) Tao, L.; Kaddis, C. S.; Loo, R. R. O.; Grover, G. N.; Loo, J. A.; Maynard, H. D. *Chem. Commun.* **2009**, 2148. (b) Tao, L.; Kaddis, C.; Ogorzalek Loo, R.; Grover, G.; Loo, J.; Maynard, H. *Macromolecules*, **2009**, DOI: 10.1021/ma901540p. (c) Henry, S.; Convertine, A.; Benoit, D.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **2009**, *20* (6), 1122.
(179) Ma, J.; Cheng, C.; Sun, G.; Wooley, K. L. *Macromolecules* **2008**, *41*, 9080.

(180) Chen, G.; Amajjahe, S.; Stenzel, M. H. *Chem. Commun.* **2009**, *10*, 1198.
(181) Valade, D.; Boyer, C.; Davis, T. P.; Bulmus, V. *Aust. J. Chem.*, DOI: 10.1071/CH09208.
(182) Gujraty, K. V.; Yanjarappa, M. J.; Saraph, A.; Joshi, A.; Mogridge, J.; Kane, R. S. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 7249.
(183) Savariar, E. N.; Thayumanavan, S. *J. Polym. Sci., Part. A: Polym. Chem.* **2004**, *42*, 6340.
(184) Relogio, P.; Charreyre, M.-T.; Farinha, J. P. S.; Martinho, J. M. G.; Pichot, C. *Polymer* **2004**, *45*, 8639.
(185) Li, Y.; Akiba, I.; Harrisson, S.; Wooley, K. L. *Adv. Funct. Mater.* **2008**, *18*, 551.
(186) Favier, A.; D'Agosto, F.; Charreyre, M.-T.; Pichot, C. *Polymer* **2004**, *45*, 7821.
(187) Li, Y. T.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81.
(188) De Lambert, B.; Chaix, C.; Charreyre, M. T.; Martin, T.; Aigoui, A.; Perrin-Rubens, A.; Pichot, C.; Mandrand, B. *Anal. Biochem.* **2008**, *373*, 229.
(189) De Lambert, B.; Chaix, C.; Charreyre, M. T.; Laurent, A.; Aigoui, A.; Perrin-Rubens, A.; Pichot, C. *Bioconjugate Chem.* **2005**, *16*, 265.
(190) Minard-Basquin, C.; Chaix, C.; D'Agosto, F.; Charreyre, M.-T.; Pichot, C. *J. Appl. Polym. Sci.* **2004**, *92*, 3784.
(191) Schilli, C. M.; Mueller, A. H. E.; Rizzardo, E.; Thang, S. H.; Chong, Y. K. *ACS Symp. Ser.* **2003**, *854*, 603.
(192) Yanjarappa, M. J.; Gujraty, K. V.; Joshi, A.; Saraph, A.; Kane, R. S. *Biomacromolecules* **2006**, *7*, 1665.
(193) Eberhardt, M.; Mruk, R.; Zentel, R.; Theato, P. *Eur. Polym. J.* **2005**, *41*, 1569.
(194) Eberhardt, M.; Theato, P. *Macromol. Rapid Commun.* **2005**, *26*, 1488.
(195) (a) Metz, N.; Theato, P. *Macromolecules* **2009**, *42*, 37. (b) Gibson, M.; Fröhlich, E.; Klok, H.-A. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 4332. (c) Boyer, C.; Davis, T. P. *Chem. Commun.*, **2009**, DOI: 10.1039/b910296e.
(196) Hwang, J.; Li, R. C.; Maynard, H. D. *J. Controlled Release* **2007**, *122*, 279.
(197) Nilles, K.; Theato, P. *Eur. Polym. J.* **2007**, *43*, 2901.
(198) Nilles, K.; Theato, P. *J. Polym. Sci., Part. A: Polym. Chem.* **2009**, *47*, 1696.
(199) Xu, J.; Zhang, W.; Zhou, N.; Zhu, J.; Cheng, Z.; Xu, Y.; Zhu, X. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 5652.
(200) Sahnoun, M.; Charreyre, M.-T.; Veron, L.; Delair, T.; D'Agosto, F. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 3551.
(201) Xiong, Q.; Ni, P.; Zhang, F.; Yu, Z. *Polym. Bull.* **2004**, *53*, 1.
(202) Broyer, R. M.; Quaker, G. M.; Maynard, H. D. *J. Am. Chem. Soc.* **2008**, *130*, 1041.
(203) Deng, Z.; Bouchekif, H.; Babooram, K.; Housni, A.; Choytun, N.; Narain, R. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4984.
(204) York, A. W.; Zhang, Y.; Holley, A. C.; Guo, Y.; Huang, F.; McCormick, C. L. *Biomacromolecules* **2009**, *10*, 936.
(205) Maki, Y.; Mori, H.; Endo, T. *Macromol. Chem. Phys.* **2007**, *208*, 2589.
(206) Boyer, C.; Loubat, C.; Robin, J. J.; Boutevin, B. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 5146.
(207) Zhang, X.; Lian, X.; Liu, L.; Zhang, J.; Zhao, H. *Macromolecules* **2008**, *41*, 7863.
(208) Jiang, X.; Zhang, J.; Zhou, Y.; Xu, J.; Liu, S. *J. Polym. Sci., Part. A: Polym. Chem.* **2008**, *46*, 860.
(209) Li, Y.; Yang, J.; Benicewicz, B. C. *J. Polym. Sci., Part. A: Polym. Chem.* **2007**, *45*, 4300.
(210) Roy, D.; Cambre, J. N.; Sumerlin, B. S. *Chem. Commun.* **2008**, 2477.
(211) Cambre, J. N.; Roy, D.; Gondi, S. R.; Sumerlin, B. S. *J. Am. Chem. Soc.* **2007**, *129*, 10348.
(212) Rossi, N. A. A.; Zou, Y.; Scott, M. D.; Kizhakkedathu, J. N. *Macromolecules* **2008**, *41*, 5272.
(213) Sun, G.; Cheng, C.; Wooley, K. L. *Macromolecules* **2007**, *40*, 793.
(214) Sun, G.; Fang, H.; Cheng, C.; Lu, P.; Zhang, K.; Walker, A. V.; Taylor, J.-S. A.; Wooley, K. L. *ACS Nano* **2009**, *3*, 673.
(215) Xiao, N. Y.; Li, A. L.; Liang, H.; Lu, J. *Macromolecules* **2008**, *41*, 2374.
(216) Zhu, J.; Di, Z.; Zhu, X.; Chen, G. *J. Polym. Sci., Part. A: Polym. Chem.* **2004**, *42*, 2558.
(217) Gudipati, C. S.; Tan, M. B. H.; Hussain, H.; Liu, Y.; He, C.; Davis, T. P. *Macromol. Rapid Commun.* **2008**, *29*, 1902.
(218) Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Discovery* **2003**, *2*, 214.
(219) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347.
(220) Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10*, 1451.
(221) Thanou, M.; Duncan, R. *Curr. Opin. Invest. Drugs (Thomson Curr. Drugs)* **2003**, *4*, 701.
(222) Fleming, C.; Maldjian, A.; Da Costa, D.; Rullay, A. K.; Haddleton, D. M.; St. John, J.; Penny, P.; Noble, R. C.; Cameron, N. R.; Davis, B. G. *Nat. Chem. Biol.* **2005**, *1*, 270.
(223) Duncan, R.; Ringsdorf, H.; Satchi-Fainaro, R. *Adv. Polym. Sci.* **2006**, *192*, 1.
(224) Satchi-Fainaro, R.; Duncan, R.; Barnes, C. M. *Adv. Polym. Sci.* **2006**, *193*, 1.
(225) Haag, R.; Kratz, F. *Angew. Chem., Int. Ed.* **2006**, *45*, 1198.
(226) Basu, A.; Yang, K.; Wang, M.; Liu, S.; Chintala, R.; Palm, T.; Zhao, H.; Peng, P.; Wu, D.; Zhang, Z.; Hua, J.; Hsieh, M.-C.; Zhou, J.; Petti, G.; Li, X.; Janjua, A.; Mendez, M.; Liu, J.; Longley, C.; Zhang, Z.; Mehlig, M.; Borowski, V.; Viswanathan, M.; Filpula, D. *Bioconjugate Chem.* **2006**, *17*, 618.
(227) Stayton, P. S.; Shimoboji, T.; Long, C.; Chilkoti, A.; Chen, G.; Harris, J. M.; Hoffman, A. S. *Nature (London, U. K.)* **1995**, *378*, 472.
(228) Hoffman, A. S. *Clin. Chem.* **2000**, *46*, 1478.
(229) Ding, Z.; Fong, R. B.; Long, C. J.; Stayton, P. S.; Hoffman, A. S. *Nature (London, U. K.)* **2001**, *411*, 59.
(230) Caliceti, P.; Veronese, F. M. *Adv. Drug Delivery Rev.* **2003**, *55*, 1261.
(231) Heredia, K. L.; Maynard, H. D. *Org. Biomol. Chem.* **2007**, *5*, 45.
(232) Thordarson, P.; Droumaguet, B.; Velonia, K. *Appl. Microbiol. Biotechnol.* **2006**, *73*, 243.
(233) Chilkoti, A.; Schwartz, B. L.; Smith, R. D.; Long, C. J.; Stayton, P. S. *Biotechnology* **1995**, *13*, 1198.
(234) Hannink, J. M.; Cornelissen, J. J. L. M.; Farrera, J. A.; Foubert, P.; De Schryver, F. C.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4732.
(235) Foubert, P.; Hannink, J. M.; Kohn, F.; Gronheid, R.; Sommerdijk, N. A. J. M.; Nolte, R. J. M.; De Feyter, S.; De Schryver, F. C. *Thin Solid Films* **2003**, *443*, 124.
(236) Hyun, J.; Lee, W.-K.; Nath, N.; Chilkoti, A.; Zauscher, S. *J. Am. Chem. Soc.* **2004**, *126*, 7330.
(237) Gotz, H.; Harth, E.; Schiller, S. M.; Frank, C. W.; Knoll, W.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 3379.
(238) Mather, B. D.; Lizotte, J. R.; Long, T. E. *Macromolecules* **2004**, *37*, 9331.
(239) Limer, A.; Haddleton, D. M. *Prog. React. Kinet. Mech.* **2004**, *29*, 187.
(240) Lecolley, F.; Tao, L.; Mantovani, G.; Durkin, I.; Lautru, S.; Haddleton, D. M. *Chem. Commun.* **2004**, 2026.
(241) Tao, L.; Mantovani, G.; Lecolley, F.; Haddleton, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 13220.
(242) Bontempo, D.; Heredia, K. L.; Fish, B. A.; Maynard, H. D. *J. Am. Chem. Soc.* **2004**, *126*, 15372.
(243) Mantovani, G.; Lecolley, F.; Tao, L.; Haddleton, D. M.; Clerx, J.; Cornelissen, J. J.; Velonia, K. *J. Am. Chem. Soc.* **2005**, *127*, 2966.
(244) Dirks, A. J. T.; Berkel, S. S. v.; Hatzakis, N. S.; Opsteen, J. A.; Delft, F. L. v.; Cornelissen, J. J. L. M.; Rowan, A. E.; Van-Hest, J. C. M.; Rutjes, F. P. J. T.; Nolte, R. J. M. *Chem. Commun.* **2005**, 4172.
(245) Bontempo, D.; Li, R. C.; Ly, T.; Brubaker, C. E.; Maynard, H. D. *Chem. Commun.* **2005**, 4702.
(246) Sumerlin, B. S.; Lowe, A. B.; Stroud, P. A.; Zhang, P.; Urban, M. W.; McCormick, C. L. *Langmuir* **2003**, *19*, 5559.
(247) Kulkarni, S.; Schilli, C.; Muller, A. H. E.; Hoffman, A. S.; Stayton, P. S. *Bioconjugate Chem.* **2004**, *15*, 747.
(248) Lai, J. T.; Shea, R. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 4298.
(249) Angot, S.; Ayres, N.; Bon, S. A. F.; Haddleton, D. M. *Macromolecules* **2001**, *34*, 768.
(250) Jakubowski, W.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 4139.
(251) Oh, J. K.; Perineau, F.; Charleux, B.; Matyjaszewski, K. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1771.
(252) Sienkowska, M. J.; Percec, V. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 635.
(253) Coelho, J. F. J.; Carvalho, E. Y.; Marques, D. S.; Popov, A. V.; Percec, V.; Gil, M. H. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6542.
(254) Coelho, J. F. J.; Silva, A. M. F. P.; Popov, A. V.; Percec, V.; Abreu, M. V.; Goncalves, P. M. O. F.; Gil, M. H. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 3001.
(255) Nguyen, N. H.; Rosen, B. M.; Lligadas, G.; Percec, V. *Macromolecules* **2009**, *42*, 2379.
(256) Lligadas, G.; Percec, V. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2745.
(257) Lligadas, G.; Ladislaw, J. S.; Guliashvili, T.; Percec, V. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *46*, 278.
(258) Lligadas, G.; Percec, V. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 4917.
(259) Percec, V.; Ramirez-Castillo, E.; Hinojosa-Falcon, L. A.; Popov, A. V. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2185.
(260) Bai, L.; Zhang, L.; Zhu, J.; Cheng, Z.; Zhu, X. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 2002.
(261) Satoh, K.; Aoshima, H.; Kamigaito, M. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 6358.

(262) Miura, Y.; Shibata, T.; Satoh, K.; Kamigaito, M.; Okamoto, Y. *J. Am. Chem. Soc.* **2006**, *128*, 16026.
(263) Baek, K.-Y.; Kamigaito, M.; Sawamoto, M. *J. Polym. Sci., Part A: Polym. Chem.* **2002**, *40*, 1937.
(264) Min, K.; Gao, H.; Matyjaszewski, K. *Macromolecules* **2007**, *40*, 1789.
(265) Thomas, D. B.; Convertine, A. J.; Myrick, L. J.; Scales, C. W.; Smith, A. E.; Lowe, A. B.; Vasilieva, Y. A.; Ayres, N.; McCormick, C. L. *Macromolecules* **2004**, *37*, 8941.
(266) Perrier, S.; Takolpuckdee, P. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 5347.
(267) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81.
(268) Lokitz, B. S.; Convertine, A. J.; Ezell, R. G.; Heidenreich, A.; Li, Y. T.; McCormick, C. L. *Macromolecules* **2006**, *39*, 8594.
(269) Tsarevsky, N. V.; Matyjaszewski, K. *ACS Symp. Ser.* **2006**, *937*, 79.
(270) Tsarevsky, N. V.; Braunecker, W. A.; Brooks, S. J.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 6817.
(271) Tsarevsky, N. V.; Pintauer, T.; Matyjaszewski, K. *Macromolecules* **2004**, *37*, 9768.
(272) Lowe, A. B.; Sumerlin, B. S.; Donovan, M. S.; McCormick, C. L. *J. Am. Chem. Soc.* **2002**, *124*, 11562.
(273) Nakayama, M.; Okano, T. *Biomacromolecules* **2005**, *6*, 2320.
(274) McDowall, L.; Chen, G.; Stenzel, M. H. *Macromol. Rapid Commun.* **2008**, *29*, 1666.
(275) Tao, L.; Liu, J.; Xu, J.; Davis, T. P. *Org. Biomol. Chem.* **2009**, *7* (17), 3481.
(276) Heredia, K. L.; Bontempo, D.; Ly, T.; Byers, J. T.; Halstenberg, S.; Maynard, H. D. *J. Am. Chem. Soc.* **2005**, *127*, 16955.
(277) Bontempo, D.; Maynard, H. D. *J. Am. Chem. Soc.* **2005**, *127*, 6508.
(278) Lele, B. S.; Murata, H.; Matyjaszewski, K.; Russell, A. J. *Biomacromolecules* **2005**, *6*, 3380.
(279) Le Droumaguet, B.; Mantovani, G.; Haddleton, D. M.; Velonia, K. *J. Mater. Chem.* **2007**, *17*, 1916.
(280) Le Droumaguet, B.; Velonia, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 6263.
(281) Nicolas, J.; San Miguel, V.; Mantovani, G.; Haddleton, D. M. *Chem. Commun.* **2006**, 4697.
(282) Narain, R.; Gonzales, M.; Hoffman, A. S.; Stayton, P. S.; Krishnan, K. M. *Langmuir* **2007**, *23*, 6299.
(283) Gody, G.; Boullanger, P.; Ladaviere, C.; Charreyre, M. T.; Delair, T. *Macromol. Rapid Commun.* **2008**, *29*, 511.
(284) Narain, R.; Housni, A.; Gody, G.; Boullanger, P.; Charreyre, M. T.; Delair, T. *Langmuir* **2007**, *23*, 12835.
(285) Housni, A.; Cai, H.; Liu, S.; Pun, S. H.; Narain, R. *Langmuir* **2007**, *23*, 5056.
(286) Yang, W. R.; Jaramillo, D.; Gooding, J. J.; Hibbert, D. B.; Zhang, R.; Willett, G. D.; Fisher, K. J. *Chem. Commun.* **2001**, 1982.
(287) Hentschel, J.; Borner, H. G. *J. Am. Chem. Soc.* **2006**, *128*, 14142.
(288) De, P.; Gondi, S. R.; Sumerlin, B. S. *Biomacromolecules* **2008**, *9*, 1064.
(289) Rozema, D. B.; Lewis, D. L.; Wakefield, D. H.; Wong, S. C.; Klein, J. J.; Roesch, P. L.; Bertin, S. L.; Reppen, T. W.; Chu, Q.; Blokhin, A. V.; Hagstrom, J. E.; Wolff, J. A. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 12982.
(290) Kim, S. H.; Jeong, J. H.; Lee, S. H.; Kim, S. W.; Park, T. G. *J. Controlled Release* **2006**, *116*, 123.
(291) Oishi, M.; Nagasaki, Y.; Itaka, K.; Nishiyama, N.; Kataoka, K. *J. Am. Chem. Soc.* **2005**, *127*, 1624.
(292) He, P.; Zheng, W. M.; Tucker, E. Z.; Gorman, C. B.; He, L. *Anal. Chem.* **2008**, *80*, 3633.
(293) Ladmiral, V.; Melia, E.; Haddleton, D. M. *Eur. Polym. J.* **2004**, *40*, 431.
(294) Lowe, A. B.; Sumerlin, B. S.; McCormick, C. L. *Polymer* **2003**, *44*, 6761.
(295) Albertin, L.; Stenzel, M. H.; Barner-Kowollik, C.; Davis, T. P. *Polymer* **2006**, *47*, 1011.
(296) Albertin, L.; Kohlert, C.; Stenzel, M.; Foster, L. J. R.; Davis, T. P. *Biomacromolecules* **2004**, *5*, 255.
(297) Albertin, L.; Stenzel, M. H.; Barner-Kowollik, C.; Foster, L. J. R.; Davis, T. P. *Macromolecules* **2005**, *38*, 9075.
(298) Albertin, L.; Cameron, N. R. *Macromolecules* **2007**, *40*, 6082.
(299) Lowe, A. B.; Wang, R. *Polymer* **2007**, *48*, 2221.
(300) Deng, Z.; Ahmed, M.; Narain, R. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 614.
(301) Opsteen, J. A.; van Hest, J. C. M. *Chem. Commun.* **2005**, 57.
(302) Ting, S. R. S.; Granville, A. M.; Quemener, D.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *Aust. J. Chem.* **2007**, *60*, 405.
(303) Granville, A. M.; Quemener, D.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromol. Symp.* **2007**, *255*, 81.
(304) Cameron, N. R.; Spain, S. G.; Kingham, J. A.; Weck, S.; Albertin, L.; Barker, C. A.; Battaglia, G.; Smart, T.; Blanazs, A. *Faraday Discuss.* **2008**, *139*, 359.
(305) Ramiah, V.; Matahwa, H.; Weber, W.; McLeary, J. B.; Sanderson, R. D. *Macromol. Symp.* **2007**, *255*, 70.
(306) Pasparakis, G.; Alexander, C. *Angew. Chem., Int. Ed. Engl.* **2008**, *47*, 4847.
(307) Oezyuerek, Z.; Komber, H.; Gramm, S.; Schmaljohann, D.; Mueller, A. H. E.; Voit, B. *Macromol. Chem. Phys.* **2007**, *208*, 1035.
(308) Zhang, L.; Bernard, J.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromol. Rapid Commun.* **2008**, *29*, 123.
(309) Ting, S. R. S.; Gregory, A. M.; Stenzel, M. H. *Biomacromolecules* **2009**, *10*, 342.
(310) Bernard, J.; Favier, A.; Zhang, L.; Nilasaroya, A.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Macromolecules* **2005**, *38*, 5475.
(311) Bernard, J.; Hao, X.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Biomacromolecules* **2006**, *7*, 232.
(312) Stenzel, M. H.; Zhang, L.; Huck, W. T. S. *Macromol. Rapid Commun.* **2006**, *27*, 1121.
(313) Bernard, J.; Save, M.; Arathoon, B.; Charleux, B. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 2845.
(314) Al-Bagoury, M.; Buchholz, K.; Yaacoub, E. J. *Polym. Adv. Technol.* **2007**, *18*, 313.
(315) Guo, T. Y.; Liu, P.; Zhu, J. W.; Song, M. D.; Zhang, B. H. *Biomacromolecules* **2006**, *7*, 1196.
(316) Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.
(317) Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, C.-L.; Chen, G.-F.; Chen, C.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2002**, *124*, 3508.
(318) De la Fuente, J. M.; Eaton, P.; Barrientos, A. G.; Menendez, M.; Penades, S. *J. Am. Chem. Soc.* **2005**, *127*, 6192.
(319) Hernaiz, M. J.; de la Fuente, J. M.; Barrientos, A. G.; Penades, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 1554.
(320) Toyoshima, M.; Miura, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1412.
(321) Spain, S. G.; Albertin, L.; Cameron, N. R. *Chem. Commun.* **2006**, 4198.
(322) As, H. *J. Controlled Release* **2008**, *132*, 153.
(323) Pan, H. Z.; Sima, M.; Kopeckova, P.; Wu, K.; Gao, S. Q.; Liu, J. H.; Wang, D.; Miller, S. C.; Kopecek, J. *Mol. Pharm.* **2008**, *5*, 548.
(324) Zhu, J. L.; Cheng, H.; Jin, Y.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. *J. Mater. Chem.* **2008**, *18*, 4433.
(325) Zhang, L.; Nguyen, T. L. U.; Bernard, J.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Biomacromolecules* **2007**, *8*, 2890.
(326) Stenzel, M. H.; Barner-Kowollik, C.; Davis, T. P.; Dalton, H. M. *Macromol. Biosci.* **2004**, *4*, 445.
(327) Rossi, N. A. A.; Mustafa, I.; Jackson, J. K.; Burt, H. M.; Horte, S. A.; Scott, M. D.; Kizhakkedathu, J. N. *Biomaterials* **2009**, *30*, 638.
(328) Chong, Y. K.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **2007**, *40*, 4446.
(329) Zhang, L. F.; Eisenberg, A. *Science* **1995**, *268*, 1728.
(330) Wang, X. S.; Guerin, G.; Wang, H.; Wang, Y. S.; Manners, I.; Winnik, M. A. *Science* **2007**, *317*, 644.
(331) Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C. M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143.
(332) Harada, A.; Kataoka, K. *Prog. Polym. Sci.* **2006**, *31*, 949.
(333) Meng, F. H.; Zhong, Z. Y.; Feijen, J. *Biomacromolecules* **2009**, *10*, 197.
(334) Li, Y. T.; Lokitz, B. S.; Armes, S. P.; McCormick, C. L. *Macromolecules* **2006**, *39*, 2726.
(335) Nystrom, A. M.; Bartels, J. W.; Du, W.; Wooley, K. L. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1023.
(336) Kopecek, J.; Bazilova, H. *Eur. Polym. J.* **1973**, *9*, 7.
(337) Caliceti, P.; Schiavon, O.; Veronese, F. M. *Bioconjugate Chem.* **2001**, *12*, 515.
(338) Torchilin, V. P.; Trubetskoy, V. S.; Whiteman, K. R.; Caliceti, P.; Ferruti, P.; Veronese, F. M. *J. Pharm. Sci.* **1995**, *84*, 1049.
(339) Torchilin, V. P.; Shtilman, M. I.; Trubetskoy, V. S.; Whiteman, K.; Milstein, A. M. *Biochim. Biophys. Acta, Biomembr.* **1994**, *1195*, 181.
(340) Jo, Y. S.; van der Vlies, A. J.; Gantz, J.; Antonijevic, S.; Demurtas, D.; Velluto, D.; Hubbell, J. A. *Macromolecules* **2008**, *41*, 1140.
(341) Yusa, S. I.; Fukuda, K.; Yamamoto, T.; Ishihara, K.; Morishima, Y. *Biomacromolecules* **2005**, *6*, 663.
(342) Ganta, S.; Devalapally, H.; Shahiwala, A.; Amiji, M. *J. Controlled Release* **2008**, *126*, 187.
(343) McCormick, C. L.; Sumerlin, B. S.; Lokitz, B. S.; Stempka, J. E. *Soft Matter* **2008**, *4*, 1760.
(344) Nakayama, M.; Okano, T. *J. Drug Delivery Sci. Technol.* **2006**, *16*, 35.
(345) Xu, X. W.; Smith, A. E.; Kirkland, S. E.; McCormick, C. L. *Macromolecules* **2008**, *41*, 8429.
(346) Lowe, A. B.; Wang, R.; Tiriveedhi, V.; Butko, P.; McCormick, C. L. *Macromol. Chem. Phys.* **2007**, *208*, 2339.
(347) Chan, Y.; Wong, T.; Byrne, F.; Kavallaris, M.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 1826.
(348) Hu, Y. Q.; Kim, M. S.; Kim, B. S.; Lee, D. S. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 3740.

(349) Nakayama, M.; Okano, T. *Macromolecules* **2008**, *41*, 504.
(350) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Angew. Chem., Int. Ed. Engl.* **2006**, *45*, 5792.
(351) Hales, M.; Barner-Kowollik, C.; Davis, T. P.; Stenzel, M. H. *Langmuir* **2004**, *20*, 10809.
(352) You, Y. Z.; Hong, C. Y.; Wang, W. P.; Lu, W. Q.; Pan, C. Y. *Macromolecules* **2004**, *37*, 9761.
(353) Zhu, J. L.; Zhang, X. Z.; Cheng, H.; Li, Y. Y.; Cheng, S. X.; Zhuo, R. X. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 5354.
(354) Chang, C.; Wei, H.; Quan, C. Y.; Li, Y. Y.; Liu, J.; Wang, Z. C.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46*, 3048.
(355) Xu, X. W.; Liu, C.; Huang, J. L. *J. Appl. Polym. Sci.* **2008**, *108*, 2180.
(356) Wang, H.; An, Y. L.; Huang, N.; Ma, R. J.; Li, J. B.; Shi, L. Q. *Macromol. Rapid Commun.* **2008**, *29*, 1410.
(357) Lowe, A. B.; Torres, M.; Wang, R. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 5864.
(358) Hopkins, S.; Carter, S.; MacNeil, S.; Rimmer, S. *J. Mater. Chem.* **2007**, *17*, 4022.
(359) Fernandez-Trillo, F.; Dureault, A.; Bayley, J. P. M.; van Hest, J. C. M.; Thies, J. C.; Michon, T.; Weberskirch, R.; Cameron, N. R. *Macromolecules* **2007**, *40*, 6094.
(360) Li, Y. H.; Tang, Y. F.; Yang, K.; Chen, X. P.; Lu, L. C.; Cai, Y. L. *Macromolecules* **2008**, *41*, 4597.
(361) Simnick, A. J.; Lim, D. W.; Chow, D.; Chilkoti, A. *Polym. Rev.* **2007**, *47*, 121.
(362) Skrabania, K.; Kristen, J.; Laschewsky, A.; Akdemir, O.; Hoth, A.; Lutz, J. F. *Langmuir* **2007**, *23*, 84.
(363) Schafer, F. Q.; Buettner, G. R. *Free Radical Biol. Med.* **2001**, *30*, 1191.
(364) Lokitz, B. S.; York, A. W.; Stempka, J. E.; Treat, N. D.; Li, Y. T.; Jarrett, W. L.; McCormick, C. L. *Macromolecules* **2007**, *40*, 6473.
(365) Yusa, S.; Yokoyama, Y.; Morishima, Y. *Macromolecules* **2009**, *42*, 376.
(366) Luo, Y. L.; Yao, X. J.; Yuan, J. F.; Ding, T.; Gao, Q. Y. *Colloids Surf.* **2009**, *68*, 218.
(367) Bisht, H. S.; Manickam, D. S.; You, Y. Z.; Oupicky, D. *Biomacromolecules* **2006**, *7*, 1169.
(368) You, Y. Z.; Manickam, D. S.; Zhou, Q. H.; Oupicky, D. *J. Controlled Release* **2007**, *122*, 217.
(369) O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. United States/Japan Forum on Advances in Polymer Chemistry and Their Impacts upon Society, S Lake Tahoe, CA, 2005; p 5203.
(370) Wang, X. J.; Liu, L.; Luo, Y.; Zhao, H. Y. *Langmuir* **2009**, *25*, 744.
(371) Park, K.; Lee, S.; Kang, E.; Kim, K.; Choi, K.; Kwon, I. C. *Adv. Funct. Mater.* **2009**, *19*, 1553.
(372) Such, G. K.; Tjipto, E.; Postma, A.; Johnston, A. P. R.; Caruso, F. *Nano Lett.* **2007**, *7*, 1706.
(373) Cortez, C.; Tomaskovic-Crook, E.; Johnston, A. P. R.; Scott, A. M.; Nice, E. C.; Heath, J. K.; Caruso, F. *ACS Nano* **2007**, *1*, 93.
(374) Zetterlund, P. B.; Kagawa, Y.; Okubo, M. *Chem. Rev.* **2008**, *108*, 3747.
(375) Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* **2007**, *32*, 93.
(376) Cunningham, M. F. *Prog. Polym. Sci.* **2008**, *33*, 365.
(377) Nguyen, T. L. U.; Farrugia, B.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 3256.
(378) Kirkland, S. E.; Hensarling, R. M.; McConaughy, S. D.; Guo, Y.; Jarrett, W. L.; McCormick, C. L. *Biomacromolecules* **2008**, *9*, 481.
(379) Suzuki, S.; Whittaker, M. R.; Grondahl, L.; Monteiro, M. J.; Wentrup-Byrne, E. *Biomacromolecules* **2006**, *7*, 3178.
(380) Hoffman, A. S.; Stayton, P. S.; Ei-Sayed, M. E. H.; Murthy, N.; Bulmus, V.; Lackey, C.; Cheung, C. *J. Biomed. Nanotechnol.* **2007**, *3*, 213.
(381) Henry, S. M.; El-Sayed, M. E. H.; Pirie, C. M.; Hoffman, A. S.; Stayton, P. S. *Biomacromolecules* **2006**, *7*, 2407.
(382) Yin, X.; Hoffman, A. S.; Stayton, P. S. *Biomacromolecules* **2006**, *7*, 1381.
(383) Roy, D.; Guthrie, J. T.; Perrier, S. *Macromolecules* **2005**, *38*, 10363.
(384) Roy, D.; Knapp, J. S.; Guthrie, J. T.; Perrier, S. *Biomacromolecules* **2008**, *9*, 91.
(385) Roy, D.; Guthrie, J. T.; Perrier, S. *Aust. J. Chem.* **2006**, *59*, 737.
(386) Roy, D.; Guthrie, J. T.; Perrier, S. *Soft Matter* **2008**, *4*, 145.
(387) Fleet, R.; McLeary, J. B.; Grumel, V.; Weber, W. G.; Matahwa, H.; Sanderson, R. D. *Eur. Polym. J.* **2008**, *44*, 2899.
(388) Hua, D. B.; Tang, J.; Cheng, J. X.; Deng, W. C.; Zhu, M. L. *Carbohydr. Polym.* **2008**, *73*, 98.
(389) Peng, Q.; Lu, S. Q.; Chen, D. Z.; Wu, X. Q.; Fan, P. F.; Zhong, R.; Xu, Y. W. *Macromol. Biosci.* **2007**, *7*, 1149.
(390) Wong, K. H.; Davis, T. P.; Bamer-Kowollik, C.; Stenzel, M. H. *Polymer* **2007**, *48*, 4950.
(391) Stenzel, M. H.; Davis, T. P. *Aust. J. Chem.* **2003**, *56*, 1035.
(392) Wong, K. H.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Aust. J. Chem.* **2006**, *59*, 539.
(393) Stenzel, M. H.; Davis, T. P.; Fane, A. G. *J. Mater. Chem.* **2003**, *13*, 2090.
(394) Hernandez-Guerrero, M.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Eur. Polym. J.* **2005**, *41*, 2264.
(395) Beattie, D.; Wong, K. H.; Williams, C.; Poole-Warren, L. A.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Biomacromolecules* **2006**, *7*, 1072.
(396) Hernandez-Guerrero, M.; Min, E.; Barner-Kowollik, C.; Mueller, A. H. E.; Stenzel, M. H. *J. Mater. Chem.* **2008**, *18*, 4718.
(397) Pirri, G.; Chiari, M.; Damin, F.; Meo, A. *Anal. Chem.* **2006**, *78*, 3118.
(398) Lou, X.; Lewis, M. S.; Gorman, C. B.; He, L. *Anal. Chem.* **2005**, *77*, 4698.
(399) Wischerhoff, E.; Uhlig, K.; Lankenau, A.; Boerner, H. G.; Laschewsky, A.; Duschl, C.; Lutz, J.-F. *Angew. Chem., Int. Ed.* **2008**, *47*, 5666.
(400) Lutz, J.-F.; Zarafshani, Z. *Adv. Drug Delivery Rev.* **2008**, *60*, 958.
(401) Christman, K. L.; Vazquez-Dorbatt, V.; Schopf, E.; Kolodziej, C. M.; Li, R. C.; Broyer, R. M.; Chen, Y.; Maynard, H. D. *J. Am. Chem. Soc.* **2008**, *130*, 16585.
(402) Hotchkiss, J. W.; Lowe, A. B.; Boyes, S. G. *Chem. Mater.* **2007**, *19*, 6.
(403) Duwez, A.-S.; Guillet, P.; Colard, C.; Gohy, J.-F.; Fustin, C.-A. *Macromolecules* **2006**, *39*, 2729.
(404) Boyer, C.; Whittaker, M.; Luzon, M.; Davis, T. P. *Macromolecules* **2009**, *42*, 6917.
(405) Rowe, M. D.; Thamm, D. H.; Kraft, S. L.; Boyes, S. G. *Biomacromolecules* **2009**, *10*, 983.
(406) Rowe, M. D.; Chang, C.-C.; Thamm, D. H.; Kraft, S. L.; Harmon, J. F., Jr.; Vogt, A. P.; Sumerlin, B. S.; Boyes, S. G. Langmuir, published online May 7, 2009, http://dx.doi.org/10.1021/la900730b.
(407) Jiang, X.; Ahmed, M.; Deng, Z.; Narain, R. *Bioconjugate Chem.* **2009**, *20*, 994.
(408) Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Vander Elst, L.; Muller Robert, N. *Chem. Rev.* **2008**, *108*, 2064.
(409) Aqil, A.; Vasseur, S.; Duguet, E.; Passirani, C.; Benoit, J. P.; Jerome, R.; Jerome, C. *J. Mater. Chem.* **2008**, *18*, 3352.
(410) Aqil, A.; Vasseur, S.; Duguet, E.; Passirani, C.; Benoit, J. P.; Roch, A.; Muller, R.; Jerome, R.; Jerome, C. *Eur. Polym. J.* **2008**, *44*, 3191.
(411) Shukoor, M. I.; Natalio, F.; Therese, H. A.; Tahir, M. N.; Ksenofontov, V.; Panthoefer, M.; Eberhardt, M.; Theato, P.; Schroeder, H. C.; Mueller, W. E. G.; Tremel, W. *Chem. Mater.* **2008**, *20*, 3567.
(412) Boyer, C.; Priyanto, P.; Davis, T. P.; Pisswan, D.; Bulmus, V.; Kavallaris, M.; Teoh, Y.-W.; Amal, R.; Carroll, M.; Woodward, R.; St. Pierre, T. *J. Mater. Chem.* DOI: 10.1039/b914063h.

CR9001403