Expert Opinion

The use of reversible addition fragmentation chain transfer polymerization for drug delivery systems

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Introduction: Reversible Addition Fragmentation Chain Transfer (RAFT) polymerisation is now an established tool for polymer chemists to create various polymer architectures with precise control over the molecular weight, and to install a variety of different moieties onto the polymer chain ends. Recently, there seems to be a trend of moving polymer science away from the traditional academic focussed research, to instead identifying real-world problems and how these can be solved with the aid of macromolecules.
Areas covered: This article has two themes; the synthesis of polymers for polymer therapeutics; and the design of polymer carriers for the physical encapsulation of drugs and genes, which can either be micelles, gels or other core-shell particles. The first part summarizes the avenues polymer chemists have developed by using RAFT polymerization to attach active compounds (such as drugs or proteins) to polymer chains. The second part gives an overview of the possibilities of using polymer nanocarriers (such as micelles, other core-shell nanoparticles, hydrogels and cationic polymers) for drug delivery.
Expert opinion: RAFT polymerisation seems to have endless possibilities in terms of macromolecular design, that is once the pitfalls of the process have been considered, which are based on the radical nature of the mechanism. Polymer chemists have explored many synthetic pathways in order to generate a myriad of structures, and to provide proof of concept for their ideas. However, considering the length of time it takes to get a polymer into a clinical trial, attention should be focussed on detailing the biological evaluation of these well-defined structures.

Keywords: core-shell nanoparticles, drug delivery, gene delivery, hydrogel, micelle, polymer therapeutics, RAFT polymerization

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1. Introduction

When treating diseases, there are many limitations with the required medication that need to be overcome: the systemic distribution of the drug(s), any adverse effects due to toxicity and allergic reactions, a low fraction of the drug(s) reaching the target, decomposition of the drug(s) and many others. A range of drug carriers have been developed over the last half a century in the hope of addressing these issues by providing a matrix for the drug for temporal control, but also to create a vehicle that can help deliver the drug to a specific target [1-8]. Two targeting mechanisms are typically envisaged, active and passive targeting. Passive targeting relies on the so-called enhanced permeability and retention effect, which is caused by

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Article highlights.

- Reversible addition fragmentation chain transfer (RAFT) polymerization provides easy access to a large variety of complex polymer architectures such as block co-polymers and star polymer with excellent control over molecular mass and a narrow molecular mass distribution. The choice of structures seems limitless in terms of functionality although the underlying radical mechanism of the process may sometimes hamper the success.
- So far, there is no evidence that polymers made by RAFT polymerization, which all carry a thiocarbonyl thio end-functionality, are cytotoxic, but more detailed investigations are required.
- Preparing polymer structures for polymer therapeutics seems effortless and a range of chemical pathways have been developed for the potential conjugation of proteins and drugs. However, RAFT polymerization can substantially broaden the array of polymers for polymer therapeutics, but currently only proof of concept is available and the test on therapeutically active compounds in combination with RAFT made polymers is still in its infancy.
- The strength of RAFT polymerization lies in improved tailoring of polymer carriers with a design specific for the drug. Increased possibilities in polymer carrier design stems from the robustness of the RAFT process in the presence of functional groups allowing the incorporation of building blocks such as glycopolymers, stimuli-responsive groups and cationic groups for binding of genes.
- Possibilities in molecular design are endless via RAFT polymerization, but the transition to improved drug delivery systems has yet to be explored in detail and can only be taken in a collaborative effort among polymer scientists, biologists, pharmacologists and clinicians.

This box summarizes key points contained in the article.

the leaky endothelium of tumor vasculature and the slow tumor drainage, leading to the preferred accumulation of a drug carrier within the tumor. Active targeting, in contrast, utilizes the presence of specific cell-receptors on the surface and purposely interacts with these, the intended targets.

Polymers in various guises, adopting an array of shapes, are now widely used for drug delivery purposes. While a vast amount of literature describes the protection and temporal control of low-molecular mass drugs, other therapeutics such as proteins, peptides and genes (oligonucleotides, siRNA, DNA) are increasingly being used and require specifically designed drug carriers. The traditional way of delivering drugs is via physical encapsulation within a polymer matrix. High compatibility between drug and polymer ensures a high loading capacity, with forces such as van der Waals, cohesive forces and H-bonding holding the drug in place. The drug then leaches out of the matrix due to a concentration gradient providing the body with a constant supply of the medicine. A range of excellent research groups – too many to list all of them here – have tackled different challenges in the last 30 – 40 years, using either physically encapsulated drugs or polymer therapeutics [4,7,9-15], but Helmut Ringsdorf, as one of the pioneers in this area, should be mentioned by name. His ground-breaking work on drug delivery extended beyond physical encapsulation. Ringsdorf proposed the covalent attachment of drugs to a polymer backbone along with introducing a terminal targeting functional group. What is important in this approach is that the drug is connected via a cleavable linker. This concept – the Ringsdorf Model – was the basis for a series of publications, all titled 'Pharmacologically Active Polymers', with the first one appearing in 1972 [16]. A publication by Ringsdorf and co-workers in 1984 discusses the inherent differences between bound and encapsulated drugs [17].

In addition to deciding whether to either physically encapsulate a drug or chemically bind it to a polymer backbone, scientists are also faced with the question as to what kind of a drug delivery carrier to choose. Choices need to be made with regard to the composition of the polymers, shape of the final assembled structures, molecular mass of the polymers and, if more than one polymer is used, the ratio between all the constituents present. Self-assembled systems, such as micelles and liposomes, are popular due to their resemblance to natural carriers such as viruses and their hydrophobic compartments [18,19]. Other nanoparticles can include solid polymer particles, which often have biocompatible polymers grafted on to the surfaces in order to increase circulation times. Increasingly popular in recent years are metal nanoparticles coated with a layer of polymers for simultaneous drug release and imaging [20]. The polymer can either be grafted onto the nanoparticles or coated, often using the layer-by-layer (LbL) technique [21,22]. Other drug delivery carriers include less defined structures such as gels. Gels are water-filled polymers, produced in a facile manner, which can be processed into films or particles of different size [23]. In contrast, polymers exhibiting highly ordered structures have also been adopted as proficient carriers with many groups focusing their attention on dendrimers, utilizing the definitive number of terminal groups [24,25].

It seems that the approaches to deliver drugs are limitless (**Figure 1**). Differently shaped carriers made from a wide range of polymer architectures and composed of various monomer units can lead to a myriad of options. However, it is interesting to look at the structures of polymers that are currently in clinical trials as drug delivery carriers. From this viewpoint, the variety seems limited indeed. Polymer therapeutics – the covalent attachment of drugs or proteins onto a polymer chain – is widely dominated by PEG [26]. Most examples of polymer–protein conjugates in clinical trials explore the so-called 'pegylation' approach, but other polymeric systems have been studied: poly[styrene maleic anhydride], poly[*N*-(2-hydroxypropyl) methacrylamide] (HPMA) and poly[N-vinyl-2-pyrrolidone] (PVP) present a selection of chemical systems currently undergoing investigation (**Table 1**) [26]. Also, a range of polymer drug

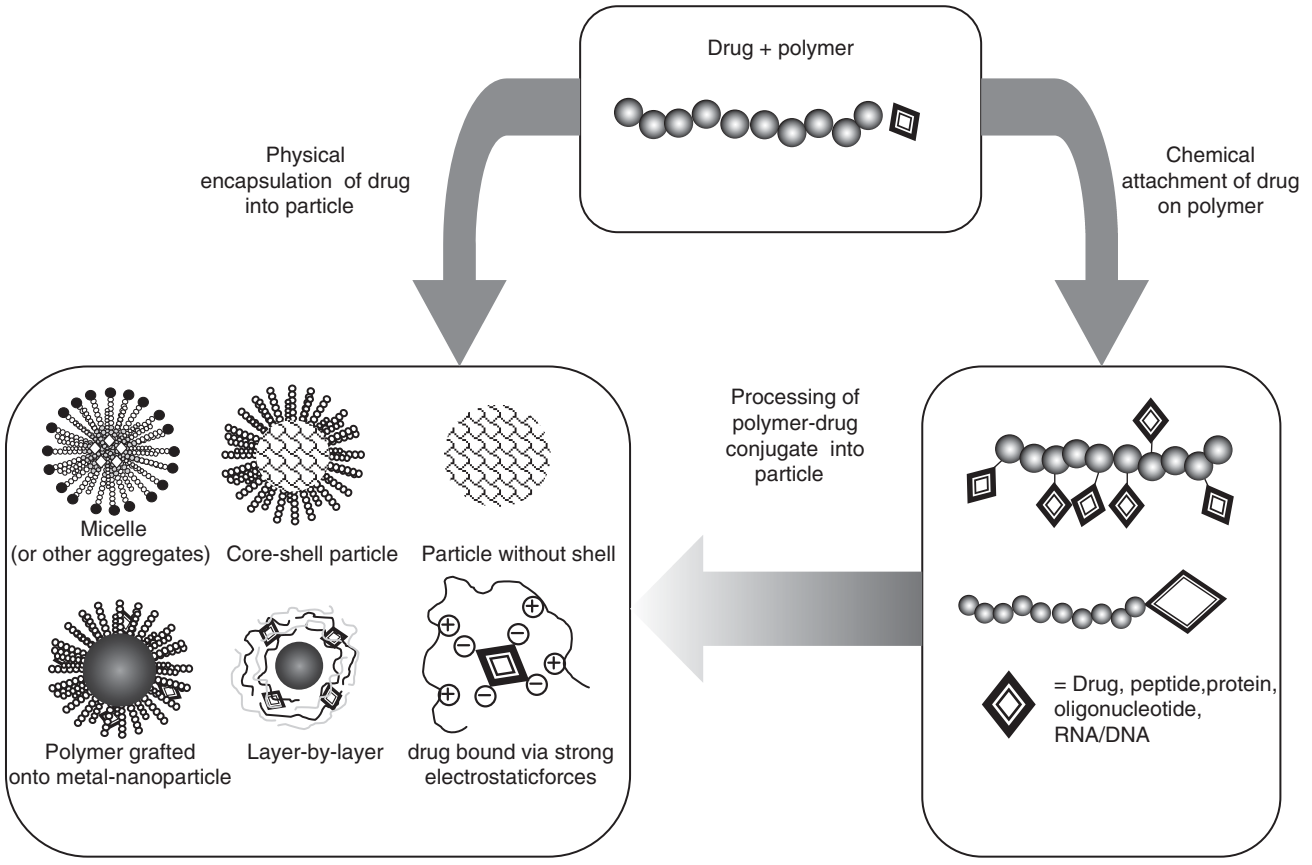


Figure 1. Avenues to the delivery of drugs and a summary of the structures discussed here.

conjugates – often in order to deliver the common anticancer drugs doxorubicin, paclitaxel and camptothecin – are being tested [26]. PEG and HPMA are in these cases accompanied by dextran and poly[L-glutamic acid] to produce carriers of interest (**Table 1**).

Drug–polymer conjugates require the presence of a hydrolytically cleavable linker between the drug and the polymer chains. One popular linker fulfilling this role is the peptide sequence Gly-Phe-Leu-Gly. In 1994, polymers based on HPMA and incorporating this linker, to bind doxorubicin, was the first synthetic polymer system to enter clinical trials [27], and still takes on the leading role in this area [15,28]. One very sophisticated polymer system, currently undergoing clinical trials, is a HPMA-based drug delivery vehicle containing the conjugated drug doxorubicin but also features targeting units of galactose to direct the vessel to the hepatocytes [29]. A range of other polymers, including a variety of block co-polymers – which are used for the physical entrapment of drugs within self-assembled structures, such as in micelles – are currently being investigated in different phases of clinical trials. **Table 1** summarizes the polymers used for the construction of micelles, which have now reached different levels of clinical evaluation [18].

Hydrogels [30-32] are more commonly used in other bio-applications, such as tissue engineering, but they possess attractive features for drug delivery devices including the viable direct implantation of cells and high biocompatibility. A selection of hydrogels are currently under preclinical and clinical trials as carriers for protein producing cells (**Table 1**) [33].

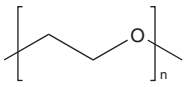
Another approach, gene therapy, utilizes cationic polymers which bind the therapeutic load via electrostatic interactions [34-36]. However, at the time of this review, with regard to reaching clinical trials and testing, only a select number viral systems and cationic surfactants are under investigation although this may change, as a cationic PLGA polymer is currently being studied for the delivery of a therapeutic DNA vaccine [37] (http://www.wiley.com/legacy/wileychi/genmed/clinical).

2. Mechanism of RAFT polymerization

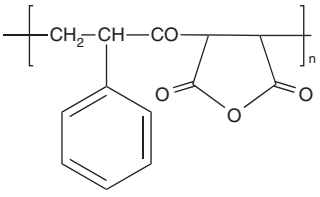
Free radical polymerization was, for an extended period of time, the preferred choice for polymerizing vinyl monomers due to the facile nature of the reactions and experimental set up. The polymerization mechanism, consisting of radical

Table 1. Examples of polymers used in clinical trials as either polymer-therapeutics or for delivery of drugs or genes.

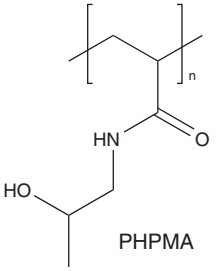
Polymers for polymer-protein conjugates



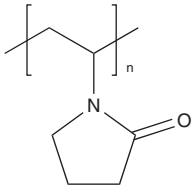
PEG



SMA

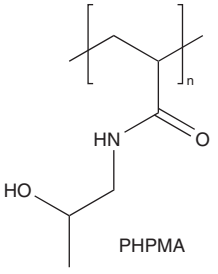


PHPMA

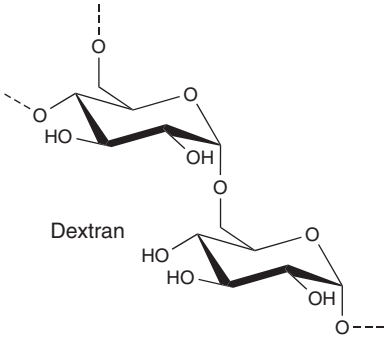


PVP

Polymers for polymer-protein conjugates



PHPMA



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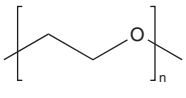
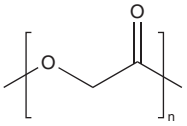
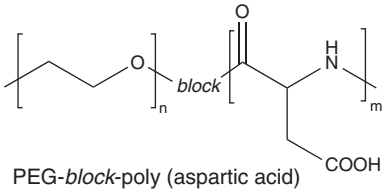


PEG

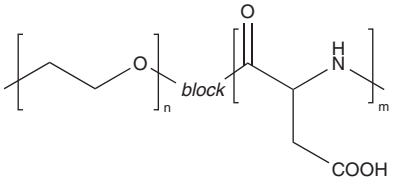


PGA

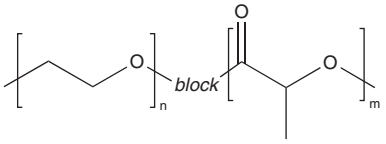


PEG-*block*-poly (aspartic acid)

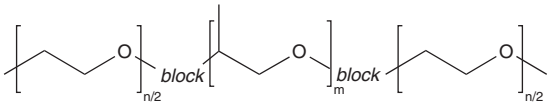
Polymers for micelles



PEG-*block*-poly(aspartic acid)



PEG-*block*-PDLLA



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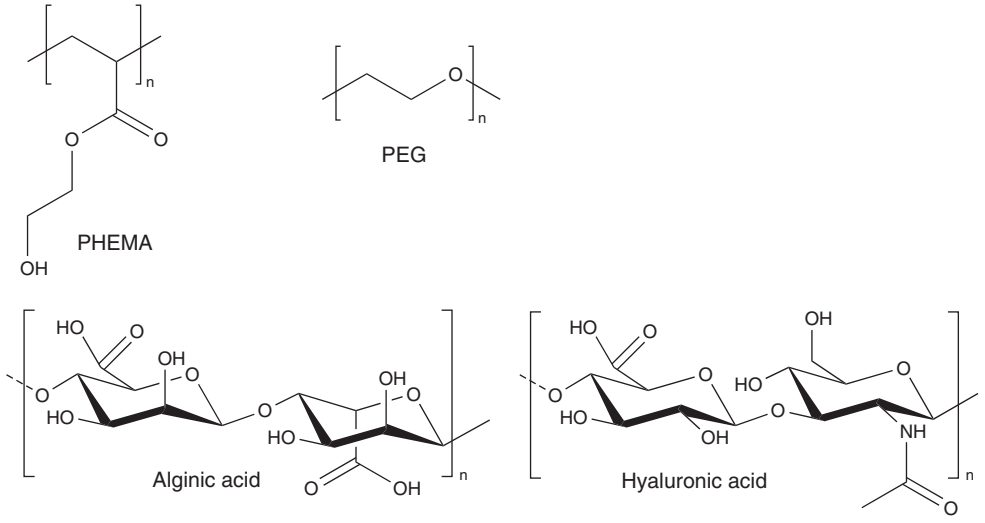
initiation, propagation and termination, allowed only limited control over molecular mass and polymer architecture. The arrival of controlled (living) radical polymerization techniques [38] overcame this drawback and the spectrum of available polymer architectures broadened. Molecular mass control and the functionalization of the termini on polymer chains can now be achieved effortlessly whilst the choice of architectures seems limitless. A range of techniques have been developed with atom transfer radical polymerization [39], nitroxide-mediated polymerization [40] and reversible addition fragmentation chain transfer (RAFT) polymerization dominating this development. Among these, RAFT polymerization [41-44] is considered one of the most versatile processes allowing the polymerization of a broad array of monomers at polymerization temperatures ranging from ambient to high temperatures.

Table 1. Examples of polymers used in clinical trials as either polymer-therapeutics or for delivery of drugs or genes (continued).

Hydrogels



Patented in 1998, the RAFT process is superimposed onto free radical polymerization [45]. The important difference with regard to conventional radical polymerization is only the addition of a so-called RAFT agent, which contains a thiocarbonyl thio functionality, to the reaction mixture. This RAFT agent plays now a vital role in controlling the process by adding at a very high rate to the radical. As soon as a radical is formed in the system via the initiation step, using thermal or photo initiation or any other radical source, ideally the radical adds to the RAFT agent much faster than it does to a new monomer unit. As a result, a semi-stable radical intermediate is formed, which prevents radical–radical termination. This intermediate eventually fragments into a new radical and a short polymer chain that carries now the thiocarbonyl thio functionality. This species is commonly termed the macroRAFT agent (**Figure 2**). In subsequent steps, this macroRAFT agent will undergo further addition–fragmentation steps and consequently the polymer will grow with monomer consumption. An important result or by-product of the RAFT process is, therefore, the presence of thiocarbonyl thio functionalities on every polymer chain end. The structure of the RAFT agent plays a central role in the polymerization. Monomers can come in all shapes and sizes and some are more reactive than others. Because of this, a variety of different RAFT agents are available, each tailored to a particular group of monomers essentially ensuring that during the polymerization the radical chain preferentially adds to a RAFT endgroup and not monomer, thereby, controlling the polymerization (**Figure 2**). Some effort was devoted to the design of the 'universal RAFT agent', a RAFT agent that is able to control the polymerization of highly reactive monomers such as vinyl acetates and at the same time slow propagating monomers [46,47]. While the RAFT process is considered a robust mechanism, there are nevertheless certain conditions that should be avoided such as: alkaline solutions, peroxides or light, which are all known to destroy the RAFT endgroup and thereby reduce the control over the growing polymer chains [48].

Whilst the detailed mechanism is subject to debate [49,50], from an application point of view only the outcome is important:

- The molecular mass increases linearly with the monomer consumption; thus, the higher the monomer conversion the higher the molecular mass.
- The more RAFT agent introduced, the smaller the molecular mass of the resulting polymer chains; therefore, the molecular mass can be adjusted according to:

$$\overline{M_n} = \frac{[M]}{[RAFT]} \times M_{monomer} \times conversion + M_{RAFT}$$

with $\overline{M_n}$ as the theoretical number-average molecular mass, [M] and [RAFT] as monomer and RAFT agent concentration at time 0, and $M_{monomer}$ and M_{RAFT} as the molecular mass (in g mol^{-1}) of the monomer and the RAFT agent, respectively.
- The polymers carry the two parts of the RAFT agent as the terminal units; consequently, the polymer chains can

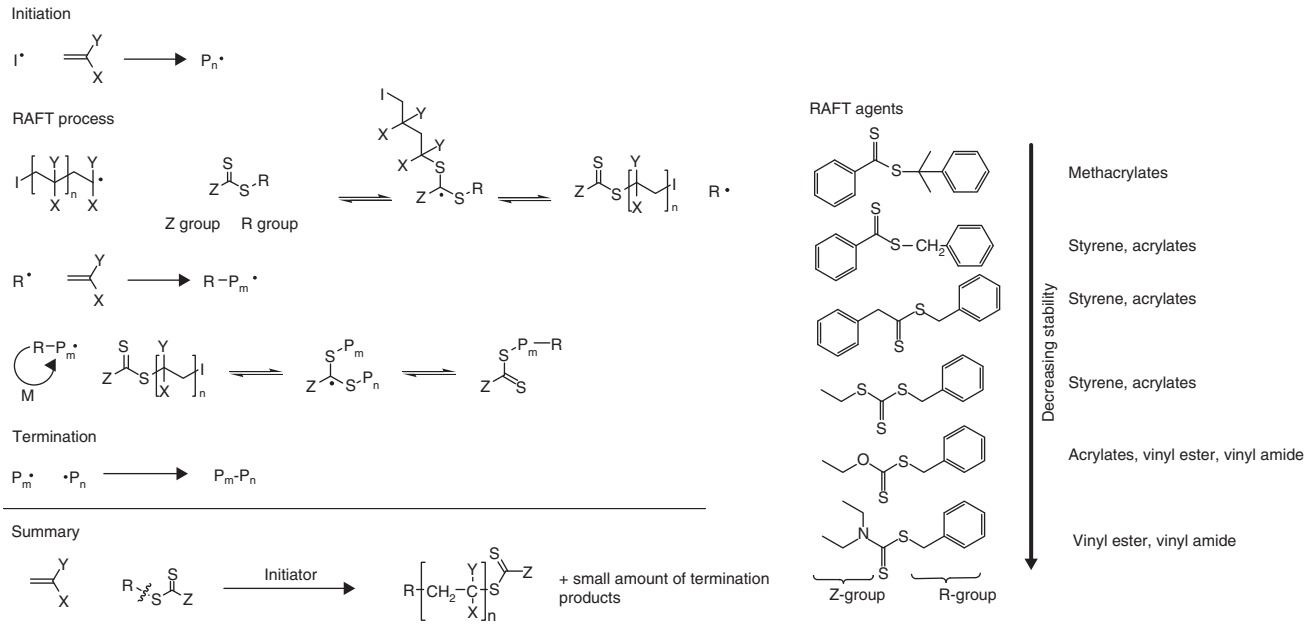


Figure 2. Mechanism of RAFT polymerization and various RAFT agents.

be functionalized theoretically at each chain end for drug conjugation.

- The molecular mass distribution is very narrow and polydispersity indices of < 1.20 are common.
- Complex architectures can be prepared. These include, but are not limited to, block co-polymers, star polymers and comb polymers (**Figure 3**).
- The polymers are typically colored either pink or yellow due to the inclusion of the thiocarbonyl thio endgroups. A loss of color typically indicates the loss of the RAFT endgroup.

The RAFT process and its applicability within polymer science are widely discussed in the literature and the reader is referred to a range of review articles [41,42,48,51-57]. The aforementioned outlined mechanism is mostly accepted, but the fine details are still contested. The reasons for effects observed during the polymerization, such as the inhibition of the polymerization in the early stages of the process and the retardation of the process compared to conventional free radical polymerization, were explained with either slow fragmentation [58,59] or cross-termination [60], although a model that combines both approaches has been developed recently [50]. From a practical standpoint, the combination of the right monomer with the right RAFT agent is the one of the fundamental decisions a scientist needs to make. **Figure 2** indicates already that different RAFT agents are necessary for different monomers. Help with this decision can be found by evaluating the review articles regarding RAFT polymerization [42,54], but commercial sources also provide succinct guidelines [61]. The reader is also advised to consider certain aspects of the process when trying to prepare complex architectures [52,62,63] such as star polymers [64] or block co-polymers [48] especially those pertaining to termination products and side reactions.

3. Toxicity of RAFT polymers

The influence of the RAFT agent on the toxicity of the polymer has frequently been questioned. The toxicity of the RAFT agent alone was tested using L929 fibroblast cell lines revealing a broad array of responses from non-toxic [65] to highly toxic [66]. However, once the RAFT agent has been incorporated into the polymer, the toxicity resulting from the endgroup is negligible. Several tests on various polymers showed that high concentrations of the macromolecules are required before any effects, arising from toxicity, can be detected [67-70]. Whilst the results may vary slightly, depending on the cell line used and the specific structure of the RAFT endgroup, concerns regarding the toxicity can be eliminated according to *in vitro* experiments. In many cases, measured toxicity was found to be the result of residual monomer or other impurities related to the synthesis of the RAFT agent or foreign contaminants unrelated to the synthetic methodologies used in the polymerization process. Despite these encouraging preliminary results, there are only initial studies on the toxicity of RAFT agents or RAFT made polymers available *in vivo* [71,72].

4. Polymer–drug conjugates

In his model, Ringsdorf proposed the covalent attachment of drugs to the polymer using a cleavable linker.

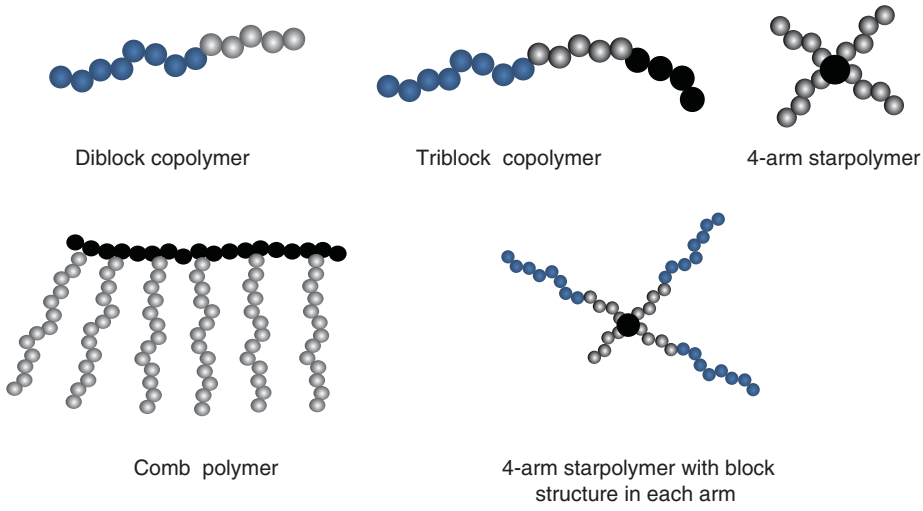


Figure 3. Examples of complex polymer architectures.

High-molecular mass drugs such as DNA or protein therapeutics are often conjugated onto the end of a polymer chain via reactions with the terminal unit on the macromolecules. In contrast, the attachment of low-molecular mass drugs often takes place along multiple reactive pendent side groups (**Figure 4**).

4.1 End-functional polymers

A highly desirable feature of RAFT polymerization is the facile access to polymers with functional terminal units. Depending on the design of the RAFT agent, telechelic polymers with functional groups on both chain ends can be obtained. In addition, the RAFT endgroups themselves can serve as functional groups for further modification.

A broad array of functional RAFT agents has been described in the literature. RAFT agents with carboxyl groups [73,74], hydroxyl groups [75], allyl groups [76], protected amines [77,78], epoxy groups [79] and many other functional RAFT agents [42,43] allow the formation of polymers with these incorporated terminal groups. Within this review, special attention is focused on the RAFT agents that have been synthesized to generate reactive polymers that can undergo conjugation to drugs, peptides, oligonucleotides and proteins. The reactive functional groups can be incorporated either via the R-group, the Z-group or indeed both groups; in the last case, this leads to the formation of telechelic polymers. Most reports focus on the synthesis of conjugates to proteins in order to create alternatives to that of the typical pegylation route [80]; other types of conjugation, in particular to peptides and RNA, have been described.

In general, three different routes are described (**Figure 5**):

i) Polymerization using a reactive RAFT agent with subsequent conjugation to the biomolecule (protein/peptide/RNA).
ii) A reaction between the RAFT agent and biomolecule(s), followed by polymerization.
iii) Conversion of the thiocarbonyl thio endgroup of a RAFT agent made polymer to a different functionality, which is suitable for further bioconjugation.

4.1.1 *End-functional polymers using functional RAFT agents for further protein/peptide conjugation*

The first report based on a reactive RAFT agent for bioconjugation focused on pyridyl disulfide (PDS) as an active endgroup for the conjugation to thiol containing proteins, such as bovine serum albumin (BSA) (**Table 2**, entry 1 – 3) [81]. A variety of PDS containing RAFT agents have since been developed, which were used to generate water soluble polymers of varying chain lengths. Depending on the structure of the RAFT agent, either simple protein conjugates, with one linear chain, or more complex architectures, such as the umbrella-like structures [82] (**Table 2**, entry 4), are accessible. One interesting focal point is the formation of α,ω-telechelic polymers containing two PDS functional groups. Once one BSA molecule has been conjugated, steric hindrance prevents further conjugation of a second BSA. This opens up the possibility for attaching an alternative, albeit, smaller protein or peptide onto the other chain end, which leads to heterotelechelic polymer conjugates [83]. The reactivity of PDS towards thiol groups was subsequently utilized in the synthesis of conjugates with peptides (**Table 2**, entry 2) [84] or siRNA (**Table 2**, entry 5) [85]. Alternatively, conjugation to the thiol groups of proteins can be carried out via maleimide functionalized polymers. The polymerization needs to be carried out using a furan-protected maleimide RAFT agent, but the final product can be easily deprotected leading to an efficient maleimide group for site-selective conjugation to free cysteines which exist in proteins (**Table 2**, entry 6) [86].

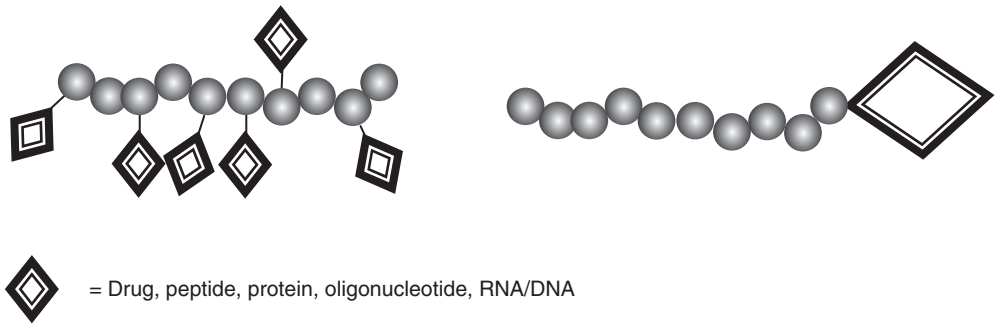


Figure 4. Polymer–drug conjugates with the drug as pendant side chain (left) or as end-functional group (right).

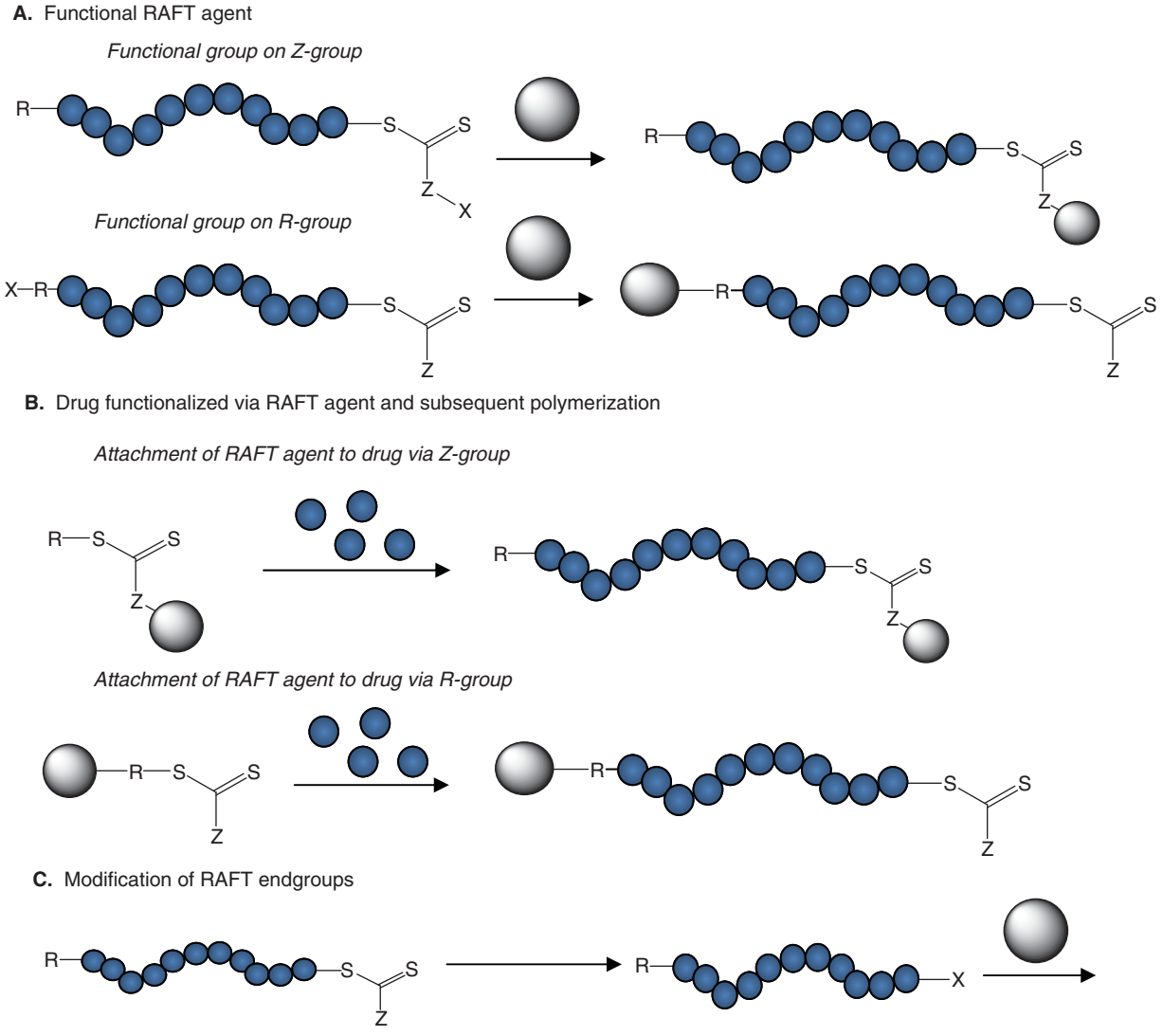
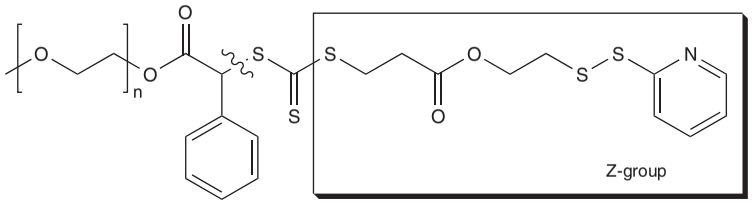
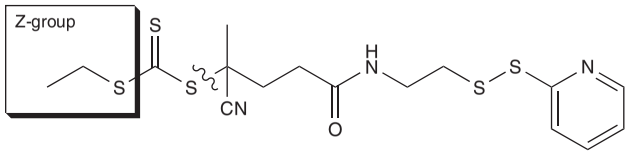
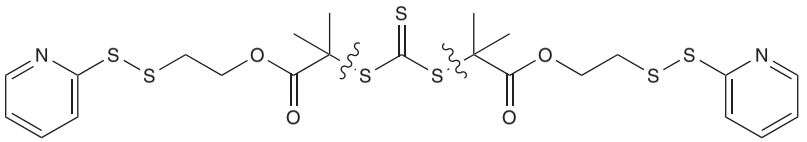
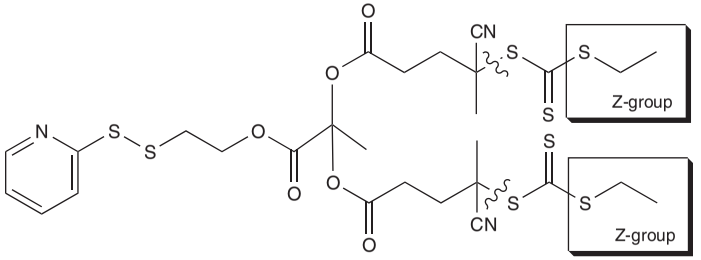
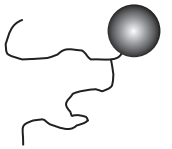
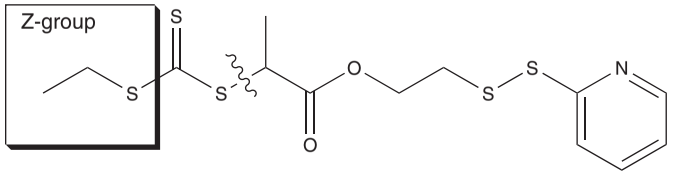


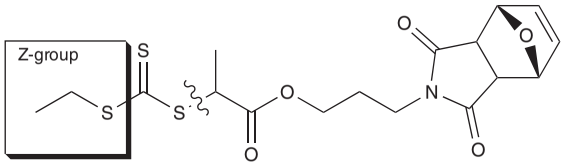
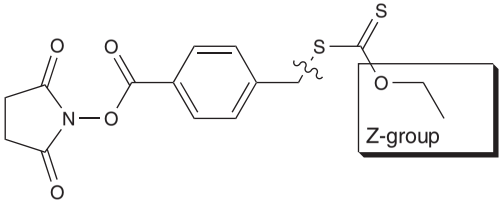
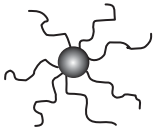
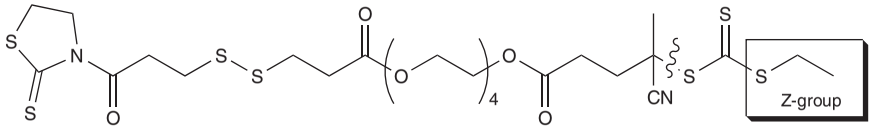
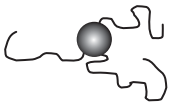
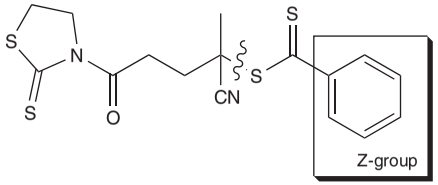
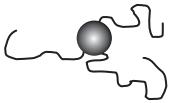
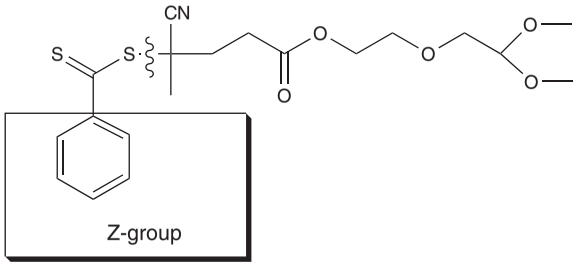
Figure 5. Different approaches to drug–polymer conjugate (drugs or genes, peptides, proteins).

Table 2. RAFT agents used to synthesize polymers for conjugation with proteins, peptides and other molecules.

	RAFT agent	Polymer	Biomolecules (reactive functional group)	Architecture
1		PEGA NIPAAm	BSA [81] (-SH)	
2		HPMA DMAEMA propylAA	Peptide (BH3) [84] (-SH)	
3		PEGA	BSA [83] (-SH)	
4		HPMA	BSA [82] (-SH)	
5		PEGA	Si-RNA [85] (-SH)	

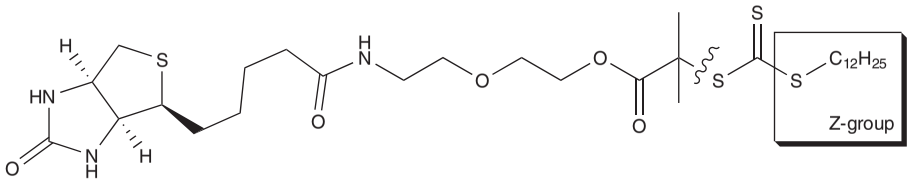
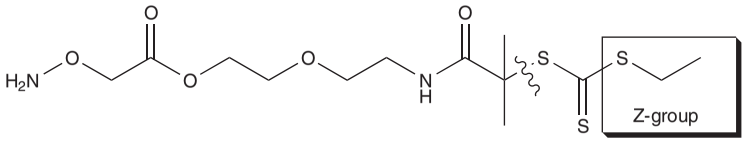
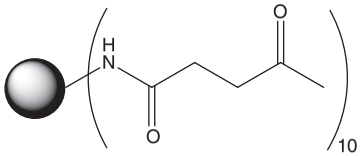
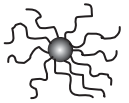
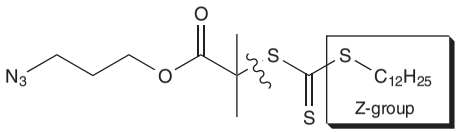
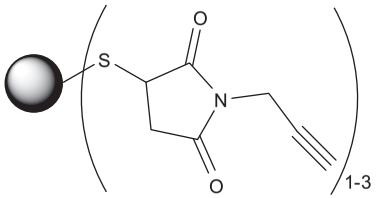
The resulting architecture is determined by the number of reactive groups in the biomolecule. The wavy line at the RAFT agent indicates the position where the monomers are inserted.
DMAEMA: *N,N*-dimethyl aminoethyl methacrylate; HPMA: 2-Hydroxypropyl methacrylamide; NIPAAm: *N*-isopropylacrylamide; PEGA: Poly (ethylene glycol) acrylate; PEGMA: Poly (ethylene glycol) methacrylate; VP: *N*-vinyl pyrrolidone.

Table 2. RAFT agents used to synthesize polymers for conjugation with proteins, peptides and other molecules (continued).

	RAFT agent	Polymer	Biomolecules (reactive functional group)	Architecture
6		PEGA	T4L [86] (-SH)	
7		VP	Lysozyme [87] ($-NH_2$)	
8		PEGMA	Lysozyme [90] ($-NH_2$)	
9		PEGMA	Lysozyme [89] ($-NH_2$)	
10		PEGMA	Peptide-RGD [88,169] ($-NH_2$)	

The resulting architecture is determined by the number of reactive groups in the biomolecule. The wavy line at the RAFT agent indicates the position where the monomers are inserted.
DMAEMA: *N,N*-dimethyl aminoethyl methacrylate; HPMA: 2-Hydroxypropyl methacrylamide; NIPAAm: *N*-isopropylacrylamide; PEGA: Poly (ethylene glycol) acrylate; PEGMA: Poly (ethylene glycol) methacrylate; VP: *N*-vinyl pyrrolidone.

Table 2. RAFT agents used to synthesize polymers for conjugation with proteins, peptides and other molecules (continued).

	RAFT agent	Polymer	Biomolecules (reactive functional group)	Architecture
11		NIPAAm, HPMA	Steptavidin [96]	
12		NIPAAm	BSA [97] 	
13		NIPAAm	BSA [246] 	

The resulting architecture is determined by the number of reactive groups in the biomolecule. The wavy line at the RAFT agent indicates the position where the monomers are inserted.
DMAEMA: *N,N*-dimethyl aminoethyl methacrylate; HPMA: 2-Hydroxypropyl methacrylamide; NIPAAm: *N*-isopropylacrylamide; PEGA: Poly (ethylene glycol) acrylate; PEGMA: Poly (ethylene glycol) methacrylate; VP: *N*-vinyl pyrrolidone.

Other popular anchor points are amine groups, which are abundant in amino acids, for example, lysine. The chemistry ranges from RAFT agents with *N*-succinimidyl esters (**Table 2**, entry 7) [87], aldehydes (**Table 2**, entry 10) [88] to RAFT agents with thiazolidine-2-thione present (**Table 2**, entry 8 – 9) as a reactive functional group [89]. A very elegant approach is the use of RAFT agents with in-built responsive functional groups. A reactive RAFT agent yielding both a thiazolidine-2-thione reactive group and a disulfide functionality allowed the formation of polymer–protein conjugates, which were stable in solution although, in a reductive environment, the protein and polymer quickly dissociate [90].

An old-time favorite in terms of polymer–protein conjugation is based on the stable recognition between biotin and streptavidin. With a dissociation constant K_d on the order of 10^{-15} mol/l [91,92], which is the strongest known protein–ligand interaction, biotin–avidin conjugation is widely used and proposed for a range of applications [93,94], including targeted drug delivery [95]. It is therefore, not surprising that polymer chemists became interested in biotin. One of the first reports on protein–polymer conjugates included a RAFT agent with a biotin functionality to yield α-biotin functionalized polymers (**Table 2**, entry 11) [96].

While thiols and amines are readily available in many proteins and some peptides for immediate conjugation with synthetic polymers, other conjugation techniques require the pre-functionalization of the biomolecule. For example, a Boc-protected aminooxy end-functionalized poly(*N*-isopropylacrylamide) (PNIPAAm) was synthesized via RAFT polymerization. At the same time, BSA was modified with levulinic acid to create a reactive ketone functionality on the protein (**Table 2**, entry 12) [97]. Another synthetic route for the attachment of biomolecules utilizes the colloquially known 'click chemistry' process [98-100]. The Cu(I) catalyzed azide-alkyne Huisgen cycloaddition requires the modification of proteins in order to introduce alkyne or azides moieties. The prior modification of proteins is necessary and has been shown to be feasible with the synthesis of an alkyne containing linker (**Table 2**, entry 13) [246]. However, care should be taken when polymerizing azide contains RAFT agents. Apart from the potential dangers when working with azides, certain reactive monomers can undergo Diels-Alder addition, resulting in the loss of azides; hence, not all the polymer chain ends are functionalized and yield a purification issue [101].

4.1.2 *RAFT modification of proteins/peptides and subsequent polymerization*

The polymerization of a protein, which has been modified to incorporate a RAFT agent, was first described by the CAMD group. The activity of BSA was only slightly affected by the presence of the RAFT agent (**Figure 6**). Growth of a water soluble polymer, oligo(ethylene glycol) acrylate (PEG-A) led to stable polymer–protein conjugates being produced. Interestingly, the disulfide group at the nexus between the protein and polymer could not be cleaved under reducing conditions [102]. The same procedure has been used to polymerize *N*-isopropylacrylamide (NIPAAm), resulting in a polymer with intrinsic thermo-responsive behavior. Below the so-called lower critical solution temperature (LCST), PNIPAAm is a water soluble polymer; once above the LCST, the polymer becomes insoluble. As a result, the BSA–PNIPAAm conjugate underwent reversible aggregate formation at higher temperatures. The BSA activity was shown to be well above 90%, despite the long protruding PNIPAAm chain [103]. A similar approach used a maleimide-functionalized RAFT agent to generate a macro-RAFT agent based on BSA. The subsequent polymerization with NIPAAm in the presence of BSA led to temperature responsive systems, which allowed for the environmental modulation of bioactivity [104].

A unique approach, in terms of the functionalization of biomolecules with RAFT agents, can be carried out with proteins or peptides with free thiol groups. The thiol functionality can be directly used and converted into the thiocarbonyl thio functionality, the RAFT group. So far, only peptides have been used for this approach and the resulting RAFT agents, which were based on cysteine and glutathione, were able to control the polymerization of acrylates [105].

4.1.3 *Conversion of RAFT endgroup into functionalities suitable for further conjugation*

The sensitivity of the RAFT agent to heat, oxidative agents and alkaline solutions can be a blessing in disguise. While these destructive events are unwanted during the polymerization, they can be deemed valuable after the polymerization has occurred. Transformations of the RAFT endgroup into other functional groups have now become a major research field in macromolecular design and polymer conjugations (**Figure 7**) [106]. Excessive heat can lead to the cleavage of the RAFT endgroup and the formation of double bonds, whilst heating in the presence of a thermal initiator allows for the replacement of the RAFT endgroup with an initiator fragment. If the reaction is carried out with an azo-initiator in tetrahydrofuran at elevated temperatures, under air and in the presence of triphenylphosphine, the RAFT endgroup is replaced with a simple hydroxyl group [107]. The replacement of the RAFT endgroup using a radical approach was successfully demonstrated using a furan-protected azo-initiator. In another reaction, after deprotection, maleimide functional groups were introduced at the chain ends for site-specific conjugation of V131CT4 lysozyme to the polymers to generate multimeric protein–polymer conjugates [108].

A very gentle technique to remove the RAFT endgroup is via aminolysis, which leads to the formation of polymers with thiol endgroups. The resulting thiols can then undergo a myriad of reactions, such as the fast and efficient reaction with double bonds (the thiol-ene reaction) [109,110] and triple bonds (the thiol-yne reaction) [111] or a nucleophilic substitution reaction with bromine containing compounds [112]. Care needs to be taken as the thiol endgroups can rapidly undergo disulfide formation leading to coupling between two polymer

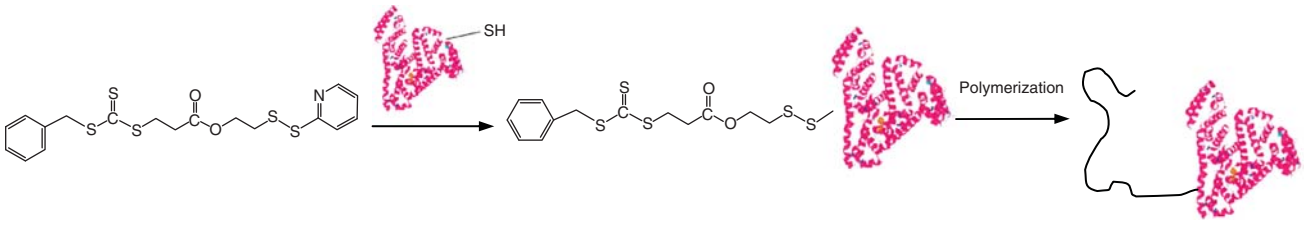


Figure 6. Modification of proteins with a RAFT agent followed by polymerization [102].

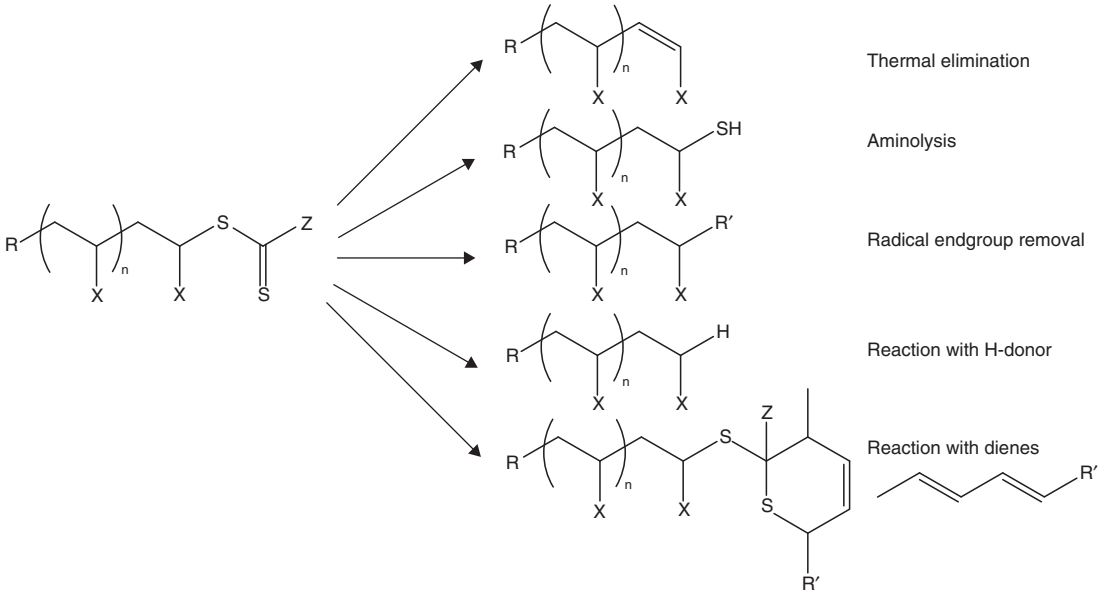


Figure 7. Transformation of RAFT endgroups [106].

chains. When this occurs, the products are twice the initial molecular mass and have a disulfide bridge present in the middle of the two chains [113]. An elegant way to prevent the formation of disulfide bridges is ensuring the presence of reactive compounds during the aminolysis reaction [114]. A versatile and useful compound for thiol endgroup stabilization is 2,2′-dithiodipyridine [115]. The resulting polymer with its PDS group can then immediately be used for protein conjugation or react with other thiol containing drugs [116]. A very unique behavior is observed when using poly(*N*-vinyl pyrrolidone) synthesized via RAFT polymerization. When left in water, the RAFT endgroup is cleaved. Surprisingly, the resulting polymer does not carry a thiol group, but a hydroxyl group instead. Heating of the polymer yields an aldehyde endgroup, which undergoes facile bioconjugation with the amine moieties found in proteins [117].

4.1.4 Complex architectures

While the sole conjugation of only one biomolecule is the most commonly reported approach, telechelic (polymers with functional groups on each chain end) bioconjugates with biomolecules on the α and ω positions have been reported. Streptavidin-BSA polymer conjugates were generated from PNIPAAm with biotin in the α position and maleimide on the ω position, which permitted further conjugation with BSA (**Figure 8**) [118]. Alternatively, two α,ω-telechelic polymers, one with azide end-functionality and one with alkyne functionality, were clicked together using Cu(I) Huisgen cycloaddition. The resulting block co-polymer carried one biotin group and one PDS group enabling further bioconjugation [119]. The conjugation of two bulky proteins is thought to be dependent on the molecular mass of the polymer chain in-between. As discussed previously, steric hindrance allows only the addition of one BSA to a telechelic polymer (**Table 2**, entry 4). This opened a window of opportunity in order to generate polymers possessing one large protein on one side of the chain, leaving the other end accessible to smaller peptides or drugs [83]. The complexity of polymer–protein conjugates can be further increased by adopting a star-shaped architecture. For this purpose, a star-polymer based on PNIPAAm was prepared via RAFT polymerization. The four arms of the star each carried a RAFT functionality. Subsequent aminolysis and the modification of the resulting thiol endgroups (as outlined above) led to a

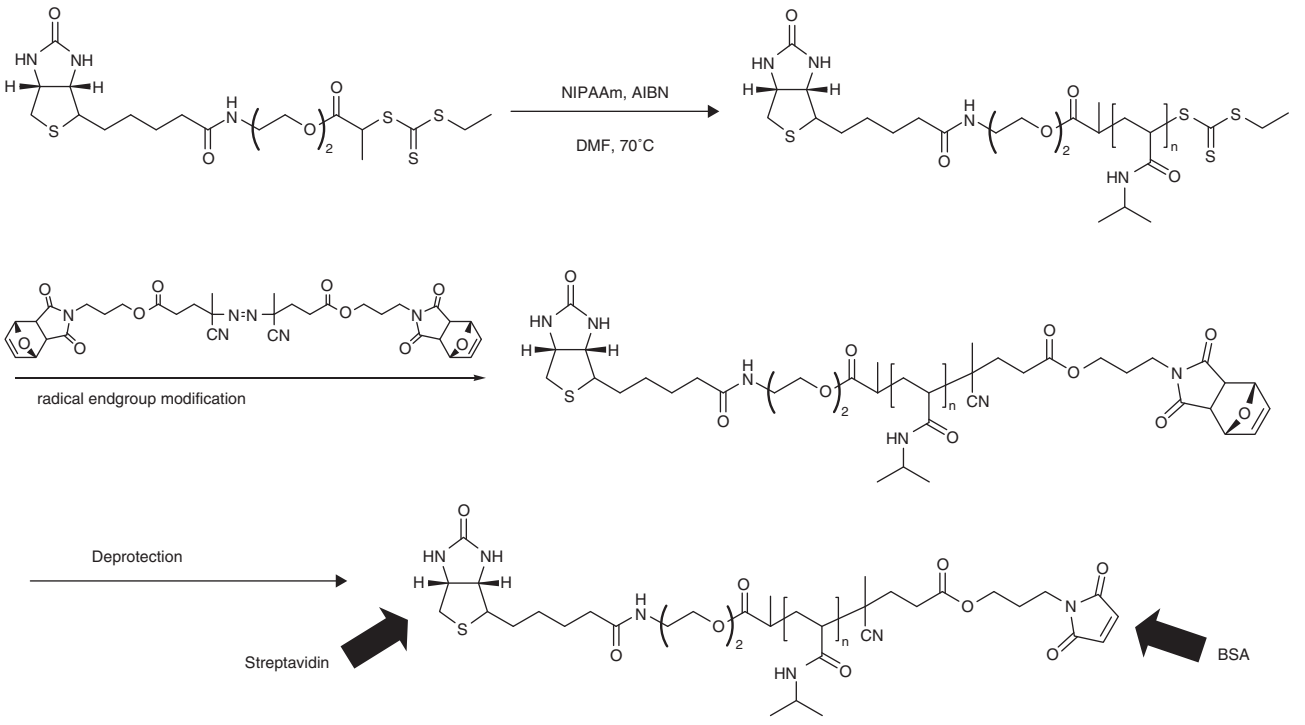


Figure 8. Synthesis of telechelic bioconjugates via RAFT endgroup modification [118].

star polymer acquiring four proteins, one at the end of each arm [108].

4.2 Reactive polymers for side chain conjugation

The end functionalization of a polymer chain is frequently only carried out to generate polymer–protein conjugates or conjugates with biomolecules of a certain size (typically large species). In contrast to this, whilst end functionalized polymers typically possess only one, or possibly two conjugated species, multiple low-molecular mass drugs, or peptides, can be connected via pendant groups present along a polymer chain. Two prerequisites are required here. The first is the possession of a functional drug that can be conjugated to the polymer chain. The second is that the polymer backbone must comprise of units that are able to connect with the functionalities present on the drug. A range of functional groups acting as pendant anchor points have been explored; these range from boronic acids [120] to aldehydes (protected as acetals) [121-124], epoxides [125], azides [126] and many others.

4.2.1 Thiol reactive monomers

The efficient reaction of the polymer with thiols [109] is desirable especially for the conjugation of polymers to peptides, but also for other thiol containing drugs. Monomers based on PDS such as PDS ethyl methacrylate can be polymerized in a controlled manner without any significant side reactions (**Table 3**, entry 1) [81,127]. The resulting polymers can either undergo fast and efficient reactions with thiols, such as glutathione [128], or can be reduced to form free thiols. This pathway was investigated to conjugate maleimide functionalized doxorubicin to the polymer [129]. PDS groups could also be introduced via post-functionalization onto a polymer chain followed by the conjugation to siRNA [130].

An increasingly popular route to conjugate thiol containing compounds onto polymers is via the thiol-alkene (thiol-ene) [110,131] or the thiol-alkyne (thiol-yne) synthetic pathway [111]. Polymers with pendant groups containing double bonds can either be obtained directly using the appropriate monomers [132-135] or via post-functionalization (**Table 3**, entry 2) [136]. A disadvantage when implementing the former is the occurrence of branching points at higher conversions, leading to gelled and crosslinked polymer products.

4.2.2 Amine reactive monomers

Drugs with amine groups can readily react with a range of functional units such as carboxylic acids. However, to achieve fast and efficient functionalization, it is advisable to choose from an array of reactive groups, for example, activated esters. The RAFT polymerization of active esters has widely been investigated. A popular method is the polymerization of *N*-acryloxysuccimide [137-141]. The resulting polymers have been reacted with a variety of amino derivatives including peptides, biotin and DNA (**Table 3**, entry 3) [142-145]. Alternatively, pentafluorophenyl(meth)acrylates [146] are used as active monomers which undergo rapid exchange with amine, forming polyamides, as demonstrated using a range of different amines (**Table 3**, entry 4) [147-150]. An elegant and

Table 3. Functional monomers used for the reaction with drugs with either thiol or amine functional groups.

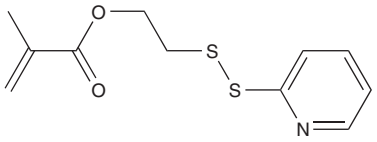
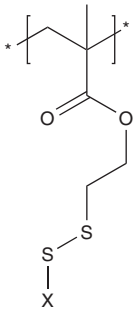
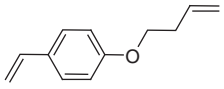
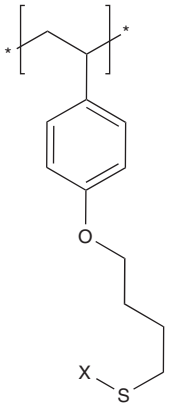
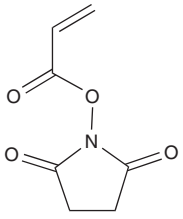
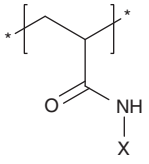
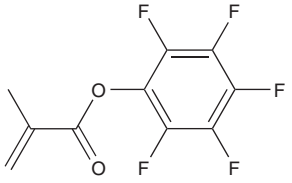
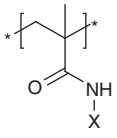
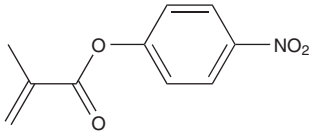
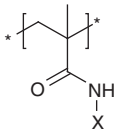
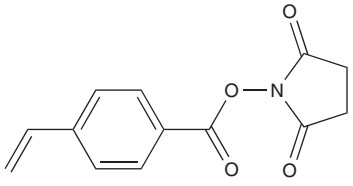
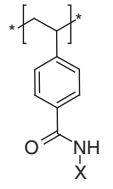
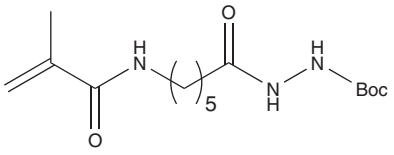
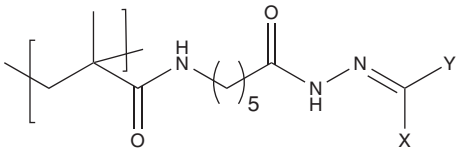
Entry	Functional group on drug	Reactive monomer	Polymer structure after functionalization
1	X-SH		
2	X-SH		
3	X-NH_2		
4	X-NH_2		
5	X-NH_2		
6	X-NH_2		

Table 3. Functional monomers used for the reaction with drugs with either thiol or amine functional groups (continued).

Entry	Functional group on drug	Reactive monomer	Polymer structure after functionalization
7	X-(C=O)-Y		

proficient approach using a one-pot procedure demonstrated that the reaction between activated esters and amines can be carried out whilst simultaneously converting the RAFT endgroup into a biotin group [151]. A range of other activated ester monomers were successfully tested regarding their ability to react with amines including *p*-nitrophenyl methacrylate (**Table 3**, entry 5) [152] and *N*-succinimide *p*-vinylbenzoate (**Table 3**, entry 6) [153]. In fact, a substantial range of 4-vinylbenzoate derivates can be used to achieve an efficient reaction with amines [154,155].

4.2.3 *Carbonyl reactive monomers*

Ketone and aldehyde reactive monomers are particularly interesting as they can potentially undergo the formation of chemical bonds, which can easily be cleaved under acidic conditions. Examples are the formation of acetals and hydrazones. Very recently, a monomer with a protected hydrazide was successfully polymerized via RAFT polymerization (**Table 3**, entry 7). Upon deprotection and subsequent conjugation to the carbonyl group of doxorubicin, a polymer–drug conjugate was created that releases the drug quickly in an acidic environment [156].

To our knowledge, the conjugation of polymers made via RAFT polymerization with low-molecular mass drugs is still in its infancy. The chemistry summarized in **Table 3** does provide many approaches to conjugate a drug to a polymer backbone although many new bonds generated are permanent such as amides. The additional introduction of a cleavable linker is, therefore, vital. Recently, the synthesis of monomers with an enzyme cleavable glycine-glycine-proline-norleucine linker connecting to the drug alendronate was reported. The subsequent RAFT polymerization yielding well-defined polymers is another example for the robustness of the RAFT process [71,72]. In this example, the polymer–drug conjugate was generated directly and no post-functionalization step was required.

5. Drug delivery systems

5.1 Micelles for drug delivery

Self-assembly of amphiphilic polymers (polymer possessing both water soluble and oil soluble sections) results in the formation of aggregates such as micelles, polymersome (vesicles), cylinders and others. The type of aggregate is usually determined by the molecular mass of polymer, and the ratio between blocks and the environmental conditions (temperature, ionic strength, pH value amongst others) [157-161]. Most frequently, amphiphilic block co-polymers are used to generate the aforementioned structures, but also other amphiphilic structures such as comb- or star polymers can be fashioned. Amongst all these aggregates, micelles are the most widely studied. The core of the aggregate is usually hydrophobic, providing an environment suitable to physically entrap hydrophobic drugs. The hydrophilic shell allows good solubility of the carrier while preventing early detection by the reticuloendothelial system due to the high hydrophilicity. The small size of these structures is an additional feature which leads to carriers with long-circulating times. Another unique facet is the potentially high surface functionality introduced by the endgroup of the block co-polymer (**Figure 9**).

The advantage of micelles prepared from amphiphilic block co-polymers, in contrast to low-molecular mass surfactants, is their increased stability as expressed by the critical micelles concentration (cmc). The higher thermodynamic stability of polymeric micelles is accompanied by a larger kinetic stability. While micelles prepared from surfactants disassemble quickly below their cmc values, polymeric micelles respond gradually to concentration changes and may maintain their shape for an extended period of time [162]. The reader is referred to an excellent review article by Allen *et al.* for further information on polymeric micelles manufactured for drug delivery purposes [159].

The sensitive correlation between the polymer structure and the resulting dynamic aggregate requires well-defined polymer architecture, primarily in terms of block length and molecular mass distribution. RAFT polymerization is an ideal technique to provide amphiphilic structures with precise molecular mass. The first block is formed by the polymerization of the monomer in the presence of the RAFT agent. In a subsequent step, this polymer is dissolved with another monomer – which will build up the second block – and the polymerization is reinitiated. The second step does not require any additional RAFT agent as the polymer prepared in the first step will act as the so-called macro-RAFT agent (**Figure 10**).

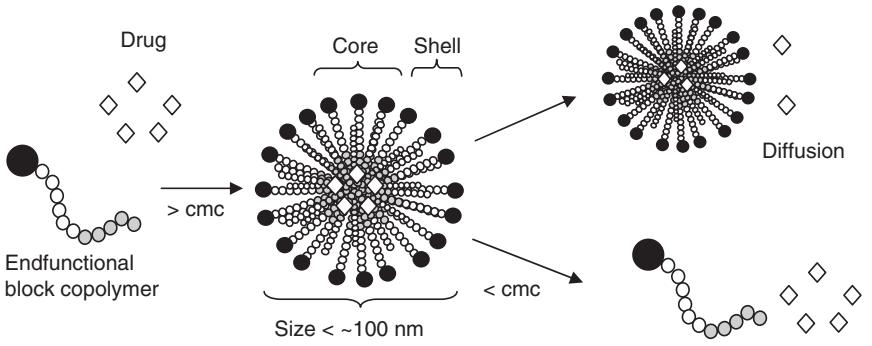


Figure 9. End-functional polymers prepared via RAFT polymerization for the self-assembly into micelles.

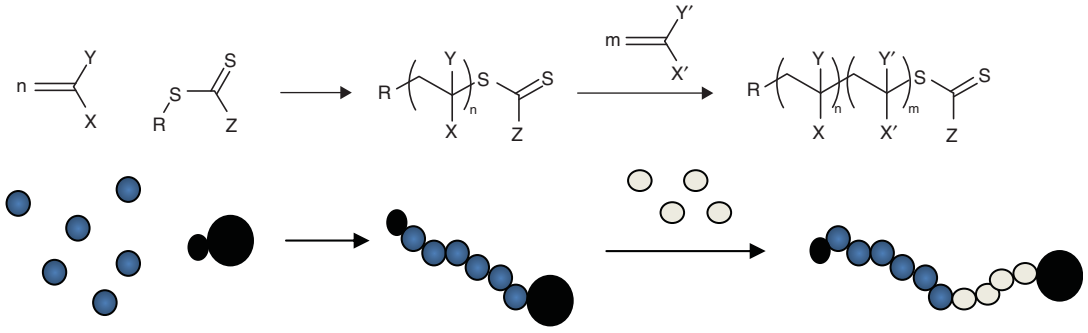


Figure 10. Block co-polymerization via RAFT polymerization.

The advantage here is the robustness of the process which takes place in the presence of many functional groups and, therefore, allows the creation of a wide variety of functional micelles. In the following section, the discussion is structured into the type of polymers which compose the water soluble block, and thus are contained within the shell-forming block and the hydrophobic units found within the core forming block.

5.2 Shell-forming block-hydrophilic polymers

5.2.1 PEG

PEG is considered the gold standard in drug delivery due to its stealth properties. As PEG cannot be prepared directly via radical polymerization, it has frequently been used as a precursor and modified with RAFT agents (for the formation of macro-RAFT agents). Subsequent polymerizations have used a range of different monomers including: butyl acrylates [163], 1,1,2,2-tetrahydrofluorodecyl acrylates [163], *N,N*-dimethyl acrylamide [164], *N*-acryloxysuccinimide [164] plus many more. By using these macro-RAFT agents, block co-polymers are formed after a single polymerization reaction. This approach was successfully applied to create PEG coated micelles for the delivery of dipyridamole [165]. A micellar system with a more complex interior consisting of cationic, as well as hydrophobic compartments but the same PEG shell, was used to deliver doxorubicin and at the same time genes [166].

Alternatively, vinyl-functionalized PEG (PEG-acrylate (PEGA), PEG methacrylate (PEGMA), methyl ether PEG-MEMA or PEGMEA (**Figure 11**)) have been deemed as viable alternatives. Polymerization of these monomers via RAFT polymerization leads to comb-like PEG structures with similar anti-fouling properties as seen with PEG alone [167]. Blocks with pendant PEG groups with molecular masses up to 100,000 g mol^{-1} can easily be prepared whilst functionalizing a single PEG chain with the same molecular mass is a difficult and daunting task. PEGMA or PEGA macro-RAFT agents were used to prepare an array of block co-polymers for the creation of micelles. Micelles prepared from block co-polymers comprised of PEGMA and styrene were shown to be readily taken up by L929 cells [168] or OVCAR cell lines [169]. The power of a biocompatible PEG shell was demonstrated using a micelle drug delivery system for oligonucleotides. The core-forming block was composed of toxic cationic polymers to bind oligonucleotides electrostatically. Shielding this core with a PEG shell led to an initially non-toxic nanoparticle that was taken up by L929 cells [170].

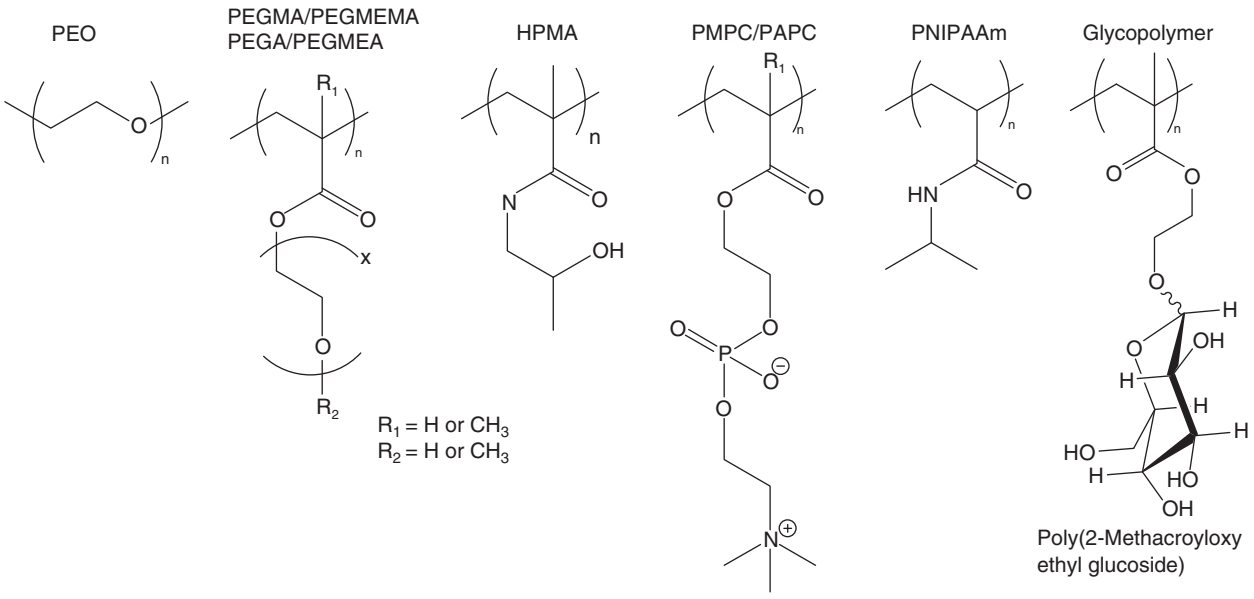


Figure 11. Examples of hydrophilic polymers synthesized via RAFT polymerization for the preparation of micelles. The glycopolymer structure given is only one example of many.

5.2.2 *N-(2-hydroxypropyl)methacrylamide*

An alternative polymer to PEG which has similar non-immunogenic and long-circulating features is poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) (**Figure 11**) [171], which can be polymerized via RAFT polymerization in a controlled manner [172]. PHPMA coated micelles were prepared from block co-polymers of HPMA and *N*-[3-(dimethylamino)propyl]methacrylamide to create a drug delivery system for siRNA [173]. Well-defined amphiphilic block co-polymers containing HPMA and thiol-reactive blocks were used to generate micelles for doxorubicin delivery [174].

5.2.3 *Poly(meth)acryloyl phosphorylcholine*

Numerous researchers have shown that surface coatings and biomaterial incorporating phosphorylcholine groups increase the biocompatibility of these synthetic materials substantially [175,176]. One of the first studies published by Ishihara *et al.* [177] showed that protein adsorption from human plasma was reduced with an increase of phosphorylcholine groups attached to the polymer. Micelles based on phosphorylcholine have been successfully prepared using RAFT polymerization [65,178]. The amphiphilic block co-polymer poly(2-methacryloyloxyethylphosphorylcholine)-*b*-poly(butyl methacrylate) (**Figure 11**) was investigated for its ability for form a micellar carrier for the drug paclitaxel [178].

5.2.4 *Stimuli-responsive polymers*

Attractive polymers for the shell of a micelle are polymers that can favorably respond to changes in the surrounding environment. Stimuli-responsive polymers usually vary their solubility turning from water soluble to insoluble or vice versa once environmental changes are applied. These alterations can be triggered by temperature [179,180], pH [181], UV light [182], oxidation [183,184] and even ultrasound [185]. These triggers can be applied externally, for example, in hyperthermia treatment, where the tumor is heated to temperatures well above 40°C, whilst other different environmental conditions can occur naturally, such as the various pH values encountered in the human body, ranging from pH 1 to 2 in the stomach, followed by pH values of above 7 in the intestine. More subtle pH changes can be found within tumors which present a slightly acidic environment with pH values of 6.75 [186]. Many regions within tumors are transiently or chronically hypoxic, and this exacerbates the tumor cells' natural tendency to overproduce acids, resulting in acidic pH values. Even healthy cells express a variety of pH values with the endocytic pathway of cells beginning near the physiological pH of 7.4, dropping to 5.5 – 6 in endosomes and approaching pH 4 – 5 in lysosomes [187,188].

Many of these stimuli-responsive polymers can be effortlessly synthesized by embracing RAFT polymerization. As previously mentioned, PNIPAAm (**Figure 11**) is a stimuli-responsive polymer that is water soluble at temperatures below its LCST, 32°C, becoming insoluble once this value has been breached. It is, therefore, not surprising that a substantial amount of effort has been expended on the RAFT polymerization of PNIPAAm for the preparation of thermo-responsive micelles [179,189-193]. Unsurprisingly, the

temperature-responsive nature of the PNIPAAm is imparted on the self-assembled micelles, which are stable at low temperatures, but start precipitating once the temperature is elevated (**Figure 12**). A similar behavior can be obtained when the shell of the micelle contains oligo(ethylene oxide) [194].

Reversible changes in solubility can also be achieved using poly(acids) or poly(bases). Poly(acids) are protonated at low pH and deprotonated at higher pH values and, therefore, highly charged, resulting in good water solubility. The opposite behavior is observed with poly(bases), which are protonated at low pH values; thus, highly water soluble. Building on this, a range of micelles has been prepared via RAFT polymerization using this reversible pH responsive concept [55,195].

5.2.5 *Glycopolymers*

Carbohydrates play a pivotal role in biological processes, such as cell–cell recognition and signaling events. The success of bacteria and viruses in their quest to invade and overrun the human body is usually derived from their carbohydrate coated surfaces. The key here is the ability of carbohydrates to connect selectively to carbohydrate-binding proteins [196]. Surprisingly, the interaction between a single sugar molecule and a protein is rather weak, but the simultaneous binding of multiple sugar molecules can result in strong conjugation. This 'glycoside cluster effect' [197] or multivalency effect is determined by the thermodynamics between the two participants but also by the architecture [198,199]. Spherical carbohydrate designs such as dendrimers with their high local carbohydrate concentrations accelerate binding to proteins. Therefore, significant effort has been targeted towards the construction of synthetic carbohydrates – glycopolymers – where the architecture and carbohydrate concentration can be tailored to achieve an optimum outcome, selective and efficient binding. The vast array of literature dedicated to the synthesis of glycopolymers [199-201] – synthetic polymers with pendant sugar groups (**Figure 11**) – via RAFT polymerization reflects the simplicity and important role of the glycopolymers. Comparative studies have been undertaking probing the glycopolymer structures and their properties. For example, an interesting observation was made when looking at self-assembled structures: micelles prepared from glycopolymer containing block co-polymers were observed to undergo strong binding to lectin, often in a superior way to analogous single linear polymer chains [202-204].

5.3 Core-forming block-hydrophobic polymers

The choice of polymer used to form the core of the micelle is directed by the type of drug that is to be incorporated within the polymeric structure. A key factor that determines the effectiveness of drug delivery in polymeric micelles is the compatibility between the drug and the hydrophobic block polymer of the micelle [205]. The term compatibility refers to the miscibility, or interaction, between the drug and the core of the micelle, without any chemical alterations taking place. In recent years, due to the timely and costly process, few groups have used the solubility parameters of polymers and drugs to estimate compatibilities. The use of solubility parameters is based on the chemistry rule: 'like dissolves like'. Therefore, a polymer is usually compatible with a drug if the intermolecular forces between both molecules are similar [206]. The total solubility parameter can be expressed as $\delta^2 = \delta_d{}^2 + \delta_p{}^2 + \delta_h{}^2$, where δ_d, δ_p and δ_h are the dispersion, polar and hydrogen bonding partial solubility parameters, respectively [207]. An example how the RAFT process can facilitate the search for a compatible polymer was demonstrated using albendazole and a random co-polymer made from methyl methacrylate (MMA) and lauryl methacrylate (LMA). According to the equation above, it was predicted that MMA should be a more suitable polymer to enhance albendazole loading whilst increasing the content of LMA should decrease loading capacity and accelerate the release of the compound. Experimental results using micelles with a core containing different MMA:LMA ratios indeed confirmed these expectations [169].

5.4 Crosslinked micelles

Micelles are known to be dynamic structures, although polymeric micelles have a higher thermodynamic and kinetic stability than low-molecular mass surfactants. Micelles can respond to changes in the environment with changes in size or even with a transition to a different morphology. Concentration changes are a key factor as these can lead to disassociation which could, in return, result in the burst-like release of drugs. To prevent premature drug release, the crosslinking of micelles is widely proposed as a suitable solution. A range of chemical reactions can be used to crosslink micelles, with the crosslinking taking place within the shell, the core or the nexus between the polymer blocks. An excellent overview comparing the different pathways can be found in reviews by Read and Armes and O'Reilly *et al.* [208,209]. Crosslinking of micelles is not unique to RAFT made polymers and the approach is only limited by the choice of functional groups. Nevertheless, there is a pathway distinctive to RAFT polymerization which is the crosslinking of micelles using a radical approach. This method is based on the fact that every block co-polymer in the micelle still carries the RAFT endgroup functionality. With the addition of divinyl compounds, crosslinking can take place capturing the micellar structure permanently. Depending on the position of the RAFT agent, core- or shell crosslinked micelles can be created [170,210-213], but, in some cases where the RAFT agent sits between the hydrophilic and hydrophobic blocks, crosslinking at the interface between the core and the shell is possible [192,214]. Whilst the crosslinking of micelles is usually conducted in a three-step procedure – synthesis of block co-polymer, self-assembly and crosslinking – the RAFT-based procedure can also be carried out in a one pot process [203,215]. The disadvantage with permanently crosslinking micelles is the substantial molecular mass of the final aggregates, which can be in excess of

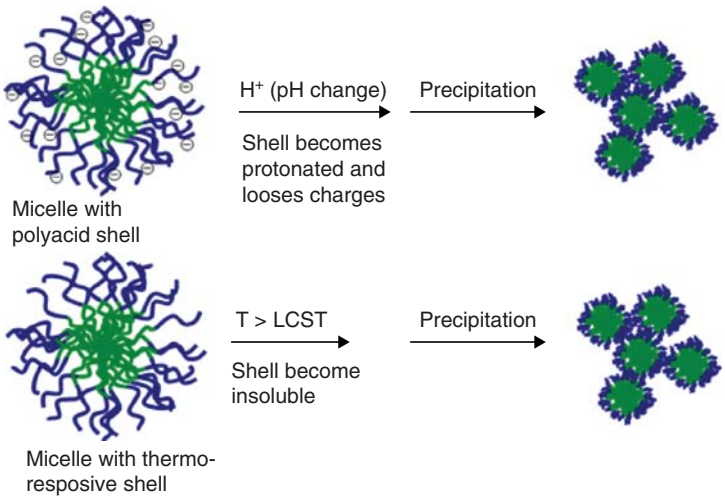


Figure 12. Thermo-responsive (bottom) and pH-responsive (top) micelles.

1,000,000 Da and prevents clearance of the drug delivery carrier after it has completed its task of releasing the contained drugs. Degradable crosslinkers utilize different pH values, or exploit the reductive environment cell interiors, to trigger the degradation of the assembled structures into the block co-polymer constituents (**Figure 13**) [211,213].

5.5 Other core-shell nanoparticles

As depicted in **Figure 1**, micelles are not the only core-shell particles that are attractive for drug delivery purposes. Alternatively, existing polymer particles can be coated with a hydrophilic shell by tethering polymer chains to the surface. A prerequisite is the presence of functional groups on the initial surface [216-218]. Many crosslinked nanoparticles, prepared via the radical polymerization of divinyl compounds, carry excess double bonds on their surface, which can be used as anchor points for a grafting through approach or for the attachment of RAFT agents for a grafting from process [66,219-221]. The thickness of the shell can then be controlled by the monomer consumption. More detailed discussions on the mechanisms involved here can be found in recent review articles [51,216]. While the grafting from approach usually leads to a thick polymer layer with brush-like structures, inherent side reaction may result in a loss of control with regard to the molecular mass of the grafted chains. An alternative tactic is to use polymers with reactive terminal functionalities which can be grafted onto the surfaces of the particles [222].

In recent years, interest has been sparked with the surface modification of metal nanoparticles, whereby applications of imaging and drug delivery can be combined. Again, RAFT polymerization was proven to be a suitable tool by allowing easy access to end functionalized polymers. The array of polymers grafted onto gold [223-228] or magnetic iron oxide nanoparticles [229-231] is steadily growing. These approaches are characterized by the covalent attachment of the polymer chain onto the various metals. LbL assembly, in contrast, is based on electrostatic interactions between charged polymers [232]. Whilst the LbL approach has been popular for many years, RAFT polymerization has been able to extend the scope of this technique by providing a versatile tool in order to generate new polymers [233-235].

5.6 Hydrogels

Hydrogels are hydrophilic polymer networks that can absorb many times their weight in water. The network of polymer can be held together by chemical crosslinking, where the chains are covalently linked with each other, or the gel is held together by physical forces such as molecular entanglements or forces such as H-bonding, ionic forces and by the interaction of built-in hydrophobic groups [236]. Due to their high water content, hydrogels are attractive for a range of applications including contact lenses and tissue engineering, but also the delivery of hydrophilic drugs such as peptides or protein-based drugs such as insulin are envisaged [237]. The RAFT process is particularly suited to synthesize hydrogel forming polymers due to the robustness of the polymerization in the presence of many functional groups, typically present in hydrogels. Despite the ease of synthesis of A-B-A triblock co-polymers to create physical networks, the number of reports on the preparation of hydrogels via RAFT is currently limited. Some selected examples based on the thermo-responsive polymer PNIPAAm are available. In one study, at temperatures above the LCST of the thermo-responsive PNIPAM blocks, associative network formation took place which led to hydrogels with mechanical properties akin to those of collagen [238]. In another example, PNIPAAm was co-polymerized with poly(acrylic acid) via RAFT polymerization. The resulting polymers underwent hydrogel formation, although this was dependent on the temperature and pH values of the solutions; observable changes in the aggregate could be seen after altering the reaction environment. The release of

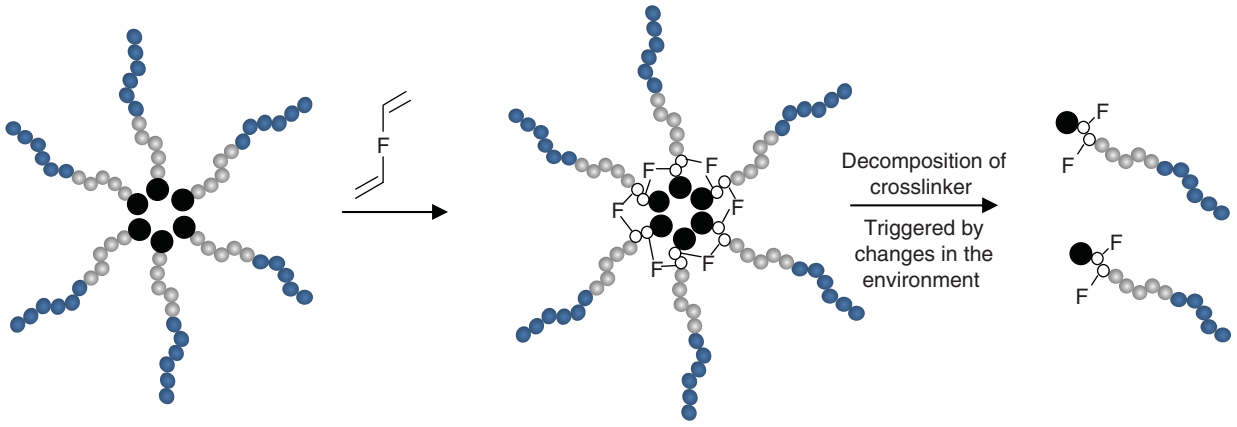


Figure 13. Crosslinking of micelles using a divinyl compound with a degradable group allowing the degradation of crosslinker in acidic or reductive conditions.

VEGF from this hydrogel could then be fine-tuned from 4 days at (pH = 7.4) to 28 days (pH = 5) [239]. A very elegant avenue to fabricate reversible hydrogels, which is very unique to the RAFT process, uses A-B-A triblock co-polymers. The key here is the utilization of a RAFT agent that, instead of being placed at the endgroup of the polymer chain, is located in the middle of the triblock co-polymer. The resulting hydrogel has a hydrophilic water-swollen matrix A, but is held together by the hydrophobic blocks B (**Figure 14**). As RAFT groups are inherently sensitive to alkaline conditions, they can be easily cleaved into thiol functionalities, thus, breaking the hydrogels into water soluble micelles. An oxidative environment can reconnect the chains by forming disulfide bridges [240].

6. Gene delivery

Gene therapy is now considered the solution to a range of diseases and genetic disorders. Gene delivery is, however, often hampered by the low stability and, therefore, the utilization of a gene carrier is expected to benefit treatment. Viral carriers are often associated with safety concerns whilst polymeric carriers are considered less toxic, have lower immunogenicity and can be tailored to the required need. Most carriers are based on cationic polymers which encapsulate DNA, RNA or oligonucleotides via electrostatic interactions. Studies have also been extended to probe and analyze other structures with testing undertaken on branched polymers, micelles and natural polymers, such as chitosan, which have a high cationic charge density at certain pH values [241].

RAFT polymerization lends itself to the synthesis of cationic polymers due to the stability of the process in the presence of functional groups. However, while permanent cationic groups do not affect the polymerization process, the presence of amines, which are protonated at low pH value, can quickly hydrolyze the RAFT agent. Nevertheless, a range of cationic polymers can be prepared for the electrostatic binding of DNA, RNA or oligonucleotides. The direct synthesis of amine containing polymers, for example, the polymerization of 2-(dimethylamino)ethyl methacrylate, amongst other amine containing monomers, has become a popular choice for RAFT chemists. Different polymer architectures, ranging from block co-polymers [170,242] for micelle formation to hyperbranched polymers [243,244], were synthesized from these monomers via the RAFT process with all of them showing good binding to DNA. As mentioned previously, the direct polymerization of these monomers can succumb to failure as the alkaline amine group can cleave the thiocarbonyl thio functionality of the RAFT agent. Careful adjustments concerning the pH value of the solutions can potentially prevent this unwanted side reaction, which coincides with the loss of the color of the RAFT agent. An alternative avenue is the post-functionalization of a pre-existing polymer. The cationic group can then be conveniently clicked onto a reactive polymer [134].

7. Conclusions

RAFT polymerization has now matured and the possibilities in terms of accessible architectures seem endless. There are only a handful of limitations and many polymer architectures, especially linear polymers including block co-polymers, can be prepared with ease. The synthesis of the more complex designs, such as star polymers, can yield some difficulties, which are often associated with the close vicinity of radicals. Most classes of monomers have successfully been polymerized and the scope of structures seems limited only by imagination. Only a handful of very reactive monomers have not been polymerized yet. As a conclusion, the RAFT process is now ready to tackle new challenges in material design, such as drug delivery. Initial reports on using RAFT polymers for drug delivery purposes are promising but a team approach with experts from other fields is needed to understand how RAFT made polymers can potentially enhance this area.

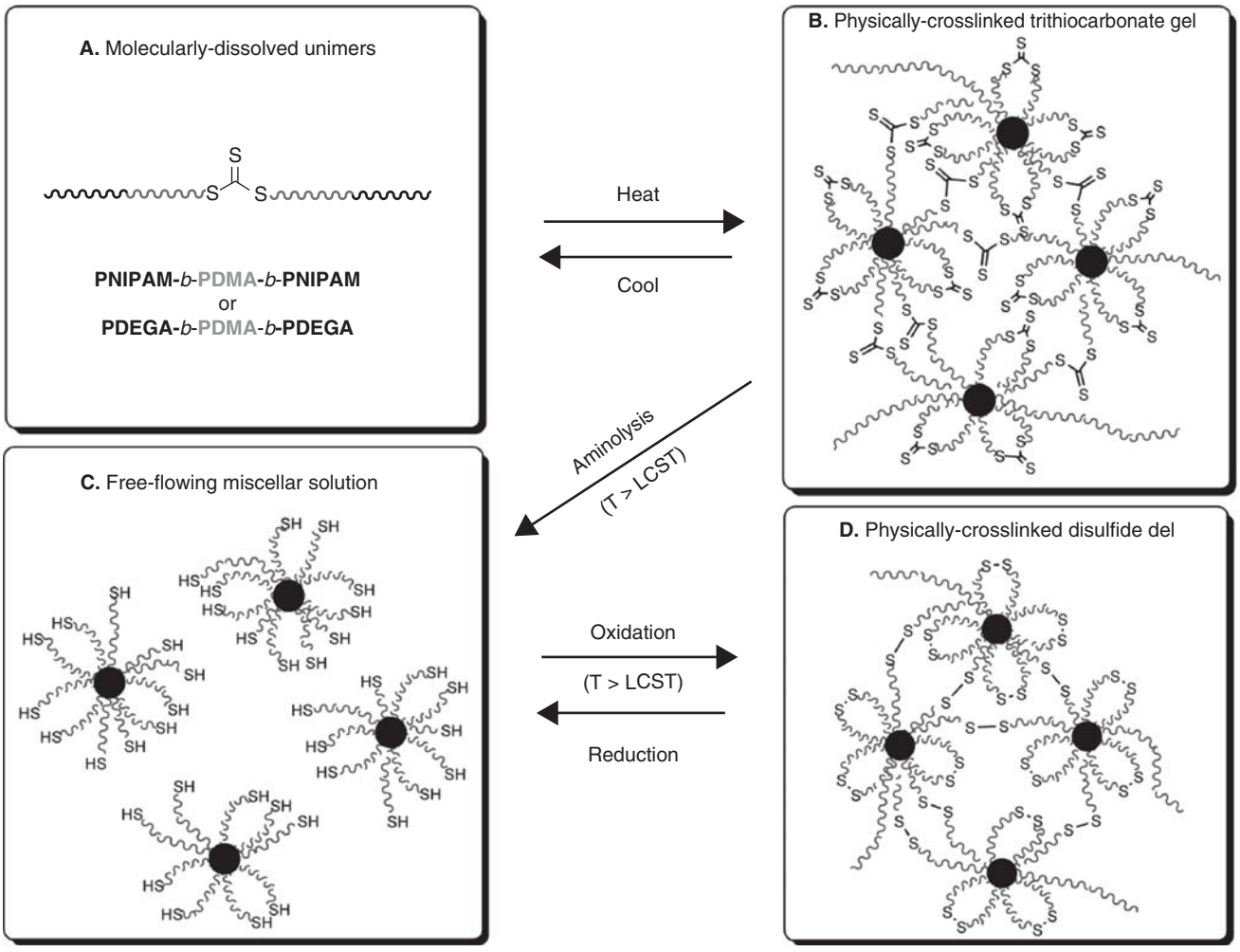


Figure 14. An example for the synthesis of hydrogels via RAFT polymerization [240].

8. Expert opinion

8.1 Application and scope of the RAFT process

• *Control over polymer structure*

It is widely proposed that RAFT polymerization can create a range of complex architectures with excellent control over the molecular mass. In reality, however, this claim is not always sustained and optimization techniques are required to achieve the best possible outcome. The reason being is that the RAFT process is based on radicals. Akin to the traditional free radical polymerization, transfer events, such as chain transfer to monomer, solvent or polymer, are always present. In addition, the RAFT process requires good access to the RAFT endgroup, which is not always given. One has to consider that a polymer is a random coil and the RAFT endgroup may be shielded inside a layer of polymer chains. This problem is magnified when more complex architectures for example, star polymers, are synthesized. Despite these side reactions, most RAFT polymerizations are reasonably successful, leading to polymers with well-defined structures and low-molecular mass distributions. The key to success is to keep the flux of radicals during the polymerization as low as possible, although not too low as to slow the polymerization down. The role of the initiator role is to start the polymerization and to control its rate but the concentration should be low enough to suppress termination events to a negligible level. There is a rule of thumb to use a molar ratio of monomer:RAFT agent:initiator of X: 1:0.1. X determines the molecular mass of the final product. If X is for example 300, the maximum number of repeating units at full conversion is 300. While in theory X can take on any value to generate polymers with very small but also very large molecular mass, in reality a very high RAFT agent concentration retards the rate of polymerization significantly, whilst a very low concentration, in an attempt to generate very large macromolecules, can lead to loss of control with broad molecular mass distributions and a large fraction of dead (terminated polymer) without any RAFT endgroup. It is important to

note that it is not only the initial initiator concentration but also the radical flux that needs to be addressed. Preferably, the small amount of initiator should decompose over a long period of time. For example, the thermal initiator AIBN (2,2′-azobisisobutyronitrile) is commonly used for polymerizations at 60°C. Significantly higher temperatures lead to a very fast decomposition of the initiator within a very short time frame; consequently, a high radical flux is present in the beginning, but the polymerization quickly slows down and stops soon after. If polymerization at higher temperature is desired, it is advised to use an initiator with a lower rate of decomposition. Also, what would happen if the amount of initiator introduced was too high? Typically, there would be an increase in the occurrence of termination reactions, which means that more polymer chains are present that do not possess a functional endgroup (for additional polymerizations or further post-modification). This result is especially detrimental when polymers need to have desired endgroups for conjugation to biomolecules, for example, proteins. The fraction of actual polymer chains with reactive endgroups will decline when the experiment is not planned carefully. Care should also be taken with regard to the RAFT agent. Light, oxidation agents (as present in many solvents such as THF), very high temperatures and an alkaline medium can potentially destroy the RAFT agent before, during and after the polymerization. This list of side reactions are only for consideration and it should not distract from the fact that the current literature is full of examples of successful ways of generating a range of polymers.

• *Range of functional groups*

The particular strength of RAFT polymerization is its versatility in terms of monomer choice. Most monomers that can be polymerized with free radical polymerization can also be polymerized using the RAFT process. Over the years, RAFT agents for most types of monomers have been developed. Successful synthetic routes have even been found for 'troublesome' monomers with amine side groups, which can potentially cleave the RAFT agent. Conducting polymerizations in buffer solutions, which protonates the amine groups, allows for controlled reactions to take place. Most functional groups, however, do not interfere with the polymerization and no special environmental conditions are required. The reader here is referred to review articles that list monomers and suitable RAFT agents.

• *Disadvantages*

At first glance, there do not seem to be many disadvantages with the RAFT process as such. It is versatile in terms of monomer choice with minimal experimental fine-tuning to provide the correct conditions, such as the right concentrations of RAFT agent and initiator, the temperature, the choice of solvent and the type of RAFT agent: these aspects require consideration but the correct choices are easily apparent after limited investigations and reading through the available literature. However, in terms of applications within drug delivery, the user of this process needs to take into account that polymers with carbon backbones are formed. These polymers are typically not degradable and the backbone can survive sometimes extreme environmental conditions. This is not to say that the side chain of the polymer cannot degrade or undergo chemical modifications. Side chain modification can potentially assist the clearance of the polymer from the body by, for example, cleaving a hydrophobic ester to a hydrophilic carboxyl group, thus, forming a fully water soluble polymer. Alternatively, polymer structures can be prepared via a combination of RAFT polymerization and other techniques such as ring-opening polymerization. The result is a polymer based on a hydrophobic degradable polyester block and a water soluble block made through RAFT polymerization. The clear advantage is that the best properties from both worlds are taken, the degradability of polyester and the high functionality and potentially water-solubility of the RAFT made polymer. Overall, researchers must not only focus on how to synthesize the polymers they need, but from an application perspective, they must also consider what happens to the drug carrier once it has delivered its payload, that is, the products produced after degradation has occurred.

8.2 The RAFT process for controlled drug delivery

• *Comparison with polymers already commercially exploited and in clinical trials*

Looking at the structures in **Table 1**, it becomes clear that only a limited selection has actually been prepared via radical polymerization. However, the structures investigated are often based on poorly defined polymers with broad molecular mass distribution. How could RAFT polymerization contribute to this field?

a) Increased structural control:

It seems that certain polymers such as PHPMA have successfully been utilized as carriers to deliver drugs. HPMA has, as outlined above, been repeatedly polymerized via RAFT polymerization with an excellent control over molecular mass, molecular mass distribution, polymer architecture and yielding macromolecules with diverse terminal functionalities. By using the monomers already approved for drug delivery and coupling them with the RAFT technique, systems can be developed that are more efficient in delivering drugs, in increased doses and with greater accuracy, that is, being released only at the intended site in the biological setting [245].

b) Exploring new structures:

While PEG is extremely popular, it may not be the only or the best option for certain applications. RAFT polymerization allows for the facile synthesis of polymers such as poly(vinyl alcohol) (from poly(vinyl acetate)) or PVP, poly(meth) acryloyl phosphorylcholine, systems which are viable alternatives to PEG with regard to performance. In addition,

new stimuli-responsive polymers such as PNIPAAm or PMAA may act as an interesting alternative and their RAFT synthesis has by now been described in many publications leading to polymers with controlled molecular mass; complex architectures such as block co-polymers and star polymers have been described. They bring with them inherent stimuli responses, feature such as pH responsiveness, which can facilitate the release of drugs in the lysozomes [245].

c) Extending the possibilities of current structures:
A range of structures in clinical trials, such as dextran or poly(lactic acid), are not derived via radical polymerization techniques. However, they can now be combined with ease with polymers made by living radical polymerization techniques. The combination of polymers made via two different techniques may account for interesting new structures such as new block co-polymers or comb polymers. Nowadays, two (or more) polymer chains can be combined from techniques that traditionally were deemed mutually exclusive, allowing for different polymeric materials, with vastly different compositions and properties, to be brought together like never before.

d) Replacing current structures with synthetic equivalents:
As mentioned repeatedly, many popular polymers such, as dextran, PEG and PLA cannot be prepared via radical polymerization. However, there are alternatives which are worthwhile considering, which can be produced via the radical route. For example, a synthetic equivalent to the carbohydrates is the glycopolymers. Although not fully degradable, glycopolymers can take on many similar functions to those of the aforementioned carbohydrates, and their bioactivity can even be increased with clever molecular design compared to their natural counterparts. While PEG is considered the 'gold standard' of drug delivery, PEGMA with its PEG side chain could be just as efficient and may even introduce added advantages derived from its umbrella-like structure. Also, poly (etherimide), an amorphous polymer structure with many branching points, is widely and successfully used for gene delivery and could be replaced by a vinyl monomer with amine side groups or any other side group that can electrostatically bind to the negatively charged gene. Advantages would be better structural control and the formation of drug carriers beyond the currently applied polyplex complexes.

• *What RAFT polymerization cannot do:*
Probably the most significant setback is the generation of polymers, which are not biodegradable. Successful attempts have been made to generate small polymer strands via RAFT and then connect them with degradable linkers, but the polymer can never fully break down. However, degradation is not always necessary and the scientist has to balance the advantages of the technique with the opposing limitations.

• *Future work*
It seems evident that there are only a few limits in terms of structural control. Scanning the literature, it seems that polymer chemists have now achieved a myriad of architectures. However, the interface of polymer science and drug delivery has only recently been explored. Initial results are promising and show that the materials are biocompatible and the structures can be easily tailored to the drug delivery needs. However, the number of reports that go beyond some initial cell experiments are limited. Many examples reported so far only provide a proof of concept that indeed it is possible to prepare potentially useful structures. At the same time, developing this kind of chemistry, optimizing polymerization kinetics and understanding side reactions is a task not to be underestimated and the achievements in this area are tremendous. However, it is now time that polymer scientists team up with clinicians, pharmacologists and biologists to explore the possibilities even further, and the knowledge obtained from years of study in their respected area now needs to be combined to advance the area of controlled drug delivery.

Declaration of interest

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