RAFT polymerization: an avenue to functional polymeric micelles for drug delivery†

Martina Heide Stenzel*

Received (in Cambridge, UK) 1st April 2008, Accepted 2nd May 2008
First published as an Advance Article on the web 5th June 2008
DOI: 10.1039/b805464a

RAFT (Reversible addition fragmentation chain transfer) polymerization is demonstrated as a versatile tool to obtain functional and crosslinked micelles for drug delivery purposes.

Polymers for controlled drug delivery

Polymers are widely investigated as carrier system for drugs in order to maintain the concentration of the drug in the body within a therapeutic window while avoiding frequent drug administrations. The target is the creation of a drug vehicle (or carrier) that allows the slow release of the drug (temporal control) or carries the drug to the site of activity (distribution control).[1] Polymer types in the focus of attention range from biodegradable polymers over hydrogels to stimuli-responsive polymers.

The advantage of polymer particles as carrier of drugs in contrast to administration of free drugs lies in the increased circulation time in the body. Fast clearance of the drug carrier is prevented since the glomerular filtration in the kidney has a molecular weight cut-off of approximately 50 000 g mol^{-1}.[2] Though, the reticuloendothelial system (RES) can detect such polymer particles eliminating them from blood circulation. Surface alteration to the particle, however, can delay or prevent recognition by the RES. Poly(ethylene glycol) is known to enhance the circulation time substantially using its high degree of hydrophilicity in order for the particles to remain undetected.[3] The clearance of the drug was also found to be considerably lowered for particles less than 200 nm, which was assigned to a higher surface curvature.[4] In addition, polymer encapsulated drugs reveal their superiority when it comes to the treatment of solid tumours. The so-called enhanced permeability and retention (EPR) effect leads to the preferred accumulation of polymers in the tumour while the ineffective lymph drainage of tumours hampers the clearance of the drug carrier.[5]

Centre for Advanced Macromolecular Design, University of New South Wales, Sydney, Australia. E-mail: M.Stenzel@unsw.edu.au; Fax: +61-2-93854344; Tel: +61-2-93856250

† Note that the term 'RAFT Polymerization' is used as a mechanistic label throughout the present contribution only. Thus, all processes that are based on reversible addition fragmentation chain transfer chemistry (including the **Ma**cromolecular **D**esign *via* the **I**nterchange of **X**anthates (MADIX) Process, see for example D. Taton, A. Z. Wilczewska, M. Destrac, *Macromol. Rapid Commun.*, 2001, **22**, 1497–1503 or M. Destrac, W. Bzducha, D. Taton, I. Gauthier-Gillaizeau, S. Z. Zard, *Macromol. Rapid Commun.*, 2002, **23**, 1049–1054) are included in this term.

Polymeric micelles as a drug delivery system

General features

It becomes clear that a polymeric carrier can further advance drug treatment. The shapes of the carrier can take up many forms. However, self-assembled structures composed from amphiphilic block copolymer (one hydrophilic, one hydrophobic block) are repeatedly mentioned as a highly promising carrier. The formed nanoparticles can take up many internal shapes and sizes depending on the nature of the block copolymer and the environmental conditions. Micelles (Fig. 1), nanospheres, nanocapsules and polymersomes (vesicles) are the main structures formed by amphiphilic block copolymers.[6] The core of the aggregate is usually hydrophobic, suitable to physically entrap hydrophobic drugs. (In contrast to hydrophobic interactions, it should be noted here, that micelles can

Martina Heide Stenzel

Martina Stenzel studied chemistry at the University of Bayreuth, Germany, before completing her PhD in 1999 at the Institute of Applied Macromolecular Chemistry, University of Stuttgart, Germany. She then received a DAAD scholarship (German Academic Exchange Service) to work as a postdoctoral Fellow at the UNESCO Centre for Membrane Science and Technology at the University of New South Wales (UNSW), Sydney, Australia. In 2002, she took on a position as a lecturer at the University of New South Wales and worked within the Centre for Advanced Macromolecular Design (CAMD) on complex polymer architectures via RAFT polymerization and on honeycomb structured porous films. She then got promoted to Senior Lecture in 2005 and Associate Professor in 2007. Her research interest is focused on the synthesis of functional polymers with complex architectures such as glycopolymers and other polymers for biomedical applications. Martina Stenzel has published more than 100 peer reviewed papers, mainly on RAFT polymerization and 3 book chapters.

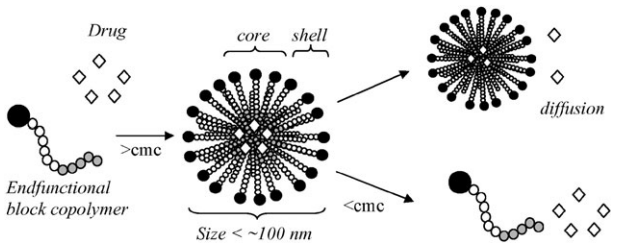

Fig. 1 Self-assembly of (functional) block copolymers into micelles above their CMC and loading and release of drugs.

also be generated using electrostatic interaction to prepare polyion complex micelles or metal complexation in the core).[7] The hydrophilic shell allows good solubility of the carrier while preventing early detection by the RES due to the high hydrophilicity. The small size of structures such as micelles is an additional feature leading to long-circulating carriers. Another unique facet is the potentially high surface functionality introduced by the end group of the block copolymer (Fig. 1).

Polymeric micelles can be obtained using a range of amphiphilic structures. Architectures can be manifold ranging from simple diblock copolymer structures to exotic dumb-bell and cyclic polymers with blocks of different polarity.[8] The diblock copolymers system is, nevertheless, the most investigated and understood system. Therefore, the focus of this feature article will concentrate on micelles derived by the self-assembly of diblock copolymers.

Block copolymer micelles have been reviewed extensively.[8,9] The most striking property is probably their dynamic structure. When block copolymers are dissolved in a selective solvent for only one block, the unimers undergo a self-organisation process similar to low molecular weight surfactants. In contrast to low molecular weight amphiphilic molecules the preparation of polymeric micelles is not as straightforward and the technique can influence the size and shape of the resulting self-assembled structure.[9] The unimer-aggregate equilibrium is then influenced by the concentration and temperature. The critical micelle concentration (CMC) and critical micelle temperature (CMT) determine the threshold of micelle formation. Micelles disintegrate into unimers below a certain concentration and above a certain temperature, which is given by the unique property of each block copolymer. In general, the CMC of a block copolymer was found to decrease with increasing block length of the core-forming hydrophobic block and with increasing overall molecular weight.[9,10] It should, however, be noted, that the stability of micelles as expressed using CMC has to be discussed from the aspect of thermodynamic and kinetic stability. The formation of micelles is determined by entropic forces. Once the structure is assembled, strong interaction between the core-forming blocks may prevent dissociation into unimers. An increased kinetic stability, which prevents immediate disintegration into unimers below the CMC, has been observed, especially with hydrophobic blocks with a high glass transition temperature. A high kinetic stability can be an important feature when the micellar drug carrier is subject to high dissolution in the blood circulation system.

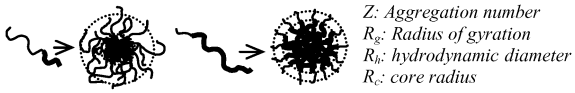

Fig. 2 Star-like micelle (left) and crew-cut micelle (right).

While the dynamics of micellization is an important consideration regarding the stability of the drug delivery system, the shape and the size of the micelle may determine the biodistribution and the drug loading capacity. Both structure parameters are usually determined by the composition of the block copolymer. As displayed in Fig. 2, star-like micelles have a corona significantly bigger than the core, while crew-cut aggregates are dominated by a substantially bigger core. Not only is the morphology of the micelle influenced by the nature of the block copolymer, but also the molecular weight of the micelle, the aggregation number Z, the hydrodynamic radius R_h and radius of gyration R_g, the size of the core R. Furthermore, the ratio R_g/R_h allows conclusion regarding the shape.

The structure and size of micelles has been subject to a range of theoretical calculations. The aim of these models is the prediction of micelle size and shape solely from the number of repeating units of both blocks N_A and N_B, or the nature of the polymer using thermodynamic approaches.[11] The scaling theory uses the size of both blocks and the interfacial tension γ to predict aggregation number Z and micelle radius.[12] The Flory-Huggins parameter χ was additionally employed in the mean-field theory to minimise the free Gibbs energy in order to obtain a correlation between N_A, N_B, χ, γ and the aggregation number Z.[13] Computer simulations such as Monte Carlo simulations were employed to predict shape and size mainly of shorter block copolymers.[14]

Despite the success of theoretical calculations to forecast structural parameters of micelles, detailed experimental studies to confirm the model cannot yet be omitted. Recent reviews[8,9,15] cover, for example, microscopy techniques (TEM), scattering techniques (SANS/SAX, SLS, DLS), fluorescence spectroscopy, gel permeation chromatography, viscosimetry surface tension measurements to investigate shape, size, chain dynamics, critical micelle concentration and kinetics of micelle formation.

One of the most important features of block copolymer micelles as drug delivery carriers is their ability to solubilize hydrophobic drugs in the hydrophobic core. This allows the solubilization of only scarcely soluble hydrophobic drugs in large quantities in an aqueous environment. The amount of drug that can be solubilized in the core, the so-called solubilization capacity, is strongly correlated to the Flory-Huggins interaction parameter χ between the solute (drug) and the polymer. The Flory-Huggins interaction parameter determines if the encapsulation of a drug is thermodynamically favourable. The amount of drug located in the micelle compared to the amount of drug dissolved in water is expressed by the partition coefficient P, which is influenced by the concentration of micelles, the CMC and the solubility of the drug in the core.[16] The Flory-Huggins interaction parameter was found to be the main parameter to control the uptake of drugs meaning that drugs with a certain polarity is best dissolved in a polymer with very similar polarity. As a result, the micelle can be quite

selective in the solubilization of solvents.[8] Encapsulation, however, is not limited to low molecular weight compounds. Even polymers can be solubilized into the core of a micelle to a limited extent.

Block copolymers for polymeric micelles

Diblock copolymers can be obtained using a range of techniques. Free radical polymerization, anionic polymerization, cationic polymerization, living/controlled radical polymerization (such as atom transfer radical polymerization (ATRP), nitroxide mediated polymerization (NMP) and reversible addition fragmentation chain transfer (RAFT)), polycondensation, ring-opening polymerization and further novel techniques are all mentioned as successful pathways to block copolymers. In addition, combination of the above mentioned techniques allows access to novel architectures. The synthesis of block copolymers has been reviewed extensively elsewhere.[8,17] Here, the focus will be on the type of polymer investigated as potential drug carrier.

Design of shell forming block

The shell of the micelle determines substantially the biocompatibility, the distribution of the drug carrier within the body and the circulation time of the micelle before being cleared. The gold standard is still poly(ethylene glycol) PEG with its stealth properties. The high hydration of the polymer chain prevents opsonin adsorption and the subsequent clearance and therefore results in long-circulating carriers.[3] The conformation of the PEG chain can greatly influence the stealth effect with longer PEG chains[18] or PEG chains tethered to a hydrophobic polymer on both sides (such as in ABA triblock copolymers)[19] showing better stability against protein adsorption. PEG has been conjugated with a range of polymers such as polypropylene glycol (pluronics©)[20] and polyesters such as poly(lactic acid),[21] poly(glycolic acid),[22] poly(ε-caprolactone)[23] or poly aminoacids[24] such as poly(aspartic acid), poly(glutamatic acid), poly(histidine) or poly(L-lysine). A range of other polymers blocks were attached by converting PEG endgroups into initiators for free radical polymerization or for controlled radical polymerization techniques.[8,25]

While the bulk of work focuses on PEG as a non-bioactive and neutral hydrophilic block with only limited response to environmental stimuli, other polymers have been explored as promising shell-forming polymers. Poly(vinyl pyrrolidone) was suggested as an alternative to PEG, however, limited synthetic procedures to obtain controlled structures have hampered the usage so far.[26] Also bio-mimicking polymers are expected to enhance greatly the circulation time of drug carriers. Polymers with phosphorylcholine side chains were found to reduce the protein adsorption from human plasma, which was related to the state of the bound water molecules in the zwitterionic structure.[27] The rise of living/controlled radical polymerization techniques offers now an avenue to obtain controlled block copolymer structures using phosphorylcholine based monomers.[28]

Stimuli-responsive features. Triggering the release of a drug once it reached its target can be seen as one of the holy grails of drug delivery. A range of polymers show sudden changes in behaviour with external stimuli like changes in pH value, temperature, ionic strength and other influences.[29,30] This can be utilized for drug delivery considering that in some biological pathways a range of pH and temperature gradients can be found.

The sudden change in solubility with temperature can be utilized for treatments where slightly increased temperatures are presented such in tumours. In addition, external stimulation such as hyperthermia treatment[31]—where the tumour is heated to temperatures well above 40 °C—cannot only result in higher blood flow to the tumour, but can also be used to cause passing drug carriers to precipitate on the heated site. The most common temperature-responsive polymer is poly(*N*-isopropyl acrylamide) PNIPAAm, which undergoes changes in solubility at an LCST of 32 °C. The LCST can be easily tailored to physiological conditions by introducing comonomers resulting in the precipitation of these polymers at elevated temperatures.[32] Several PNIPAAm based micellar systems have been prepared showing drug release set off by temperature changes.[33]

It is obvious that orally administered drugs pass areas with significantly different pH value considering that a pH value of 1–2 can be found in the stomach followed by pH values of above 7 in the intestine. More subtle pH changes can be found in tumours having a slightly acidic environment with a pH value of 6.75.[34] Many regions within tumors are transiently or chronically hypoxic, and this exacerbates 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, and then drops to a lower pH of 5.5–6.0 in endosomes and approaches pH 4.0–5.0 in lysosomes.[35] Polymers, which change their solubility within this pH range, can be employed to generate a responsive drug delivery system. Polybases and polyacids are widely investigated for drug delivery purposes. Poly(dimethyl aminoethyl methacrylate) is hydrophobic at physiological conditions, but becomes hydrophilic when protonated.[36] Similar properties can be achieved with histidine[37] or pyridine[38,39] based block copolymers. Poly(meth)acrylic acids in contrast belong to polyacids, which are typically deprotonated in alkaline conditions, thus show a better solubility in aqueous solutions than in their protonated state at low pH values.[40–43]

While these approaches using polyacids and polybases are reversible property changes, the introduction of acid-labile groups can lead to the permanent destruction of polymer structures once the carrier reached its final destination. The slightly acidic pH value in tumours or in the cell interior can catalyse the cleavage of certain functional groups such as acetals[44,45] or hydrazones,[46] therefore accelerating the drug release.

Oxidative processes can alter the structure of a polymer leading to permanently changed solubilities of micelles as it has been demonstrated using the oxidation of hydrophobic thioethers to hydrophilic sulfoxides[47] or the oxidation of ferrocene containing micelles.[48,49]

External stimuli can include UV-irradiation[30] or ultrasound[50] treatment resulting in triggered drug release.

Proteins/Peptides. While protein or peptides can act as therapeutic agents themselves, the focus here is more on protein/peptides as building blocks in amphiphilic block copolymer.

Protein/peptides can be treated as simply a water-soluble block with potentially high biocompatibility. However, more attractive is their property to interact with receptors on cell surfaces. These specific ligand–receptor interactions equip the drug carrier with a map to target cells, which overexpress these protein/peptide receptors. Several proteins or peptides are known to allow the targeted delivery of drugs such as transferrin. Transferrin receptors are abundant in different cancer cell lines.[51] Transferrin bound to amphiphilic block copolymers can therefore be a promising pathway in targeting specifically tumour cells while healthy cells remain unaffected.[52,53] Other examples include the epidermal growth factor, whose receptors are overexpressed in hepatocellular carcinoma.[54,55]

While one of the main targets in drug delivery is the selective delivery of the carrier to sites where the drug is needed, the uptake of nano carriers into the cell interior is still a major challenge. Cell-penetrating peptides[56] have been shown to enable the delivery a range of nanocarriers to different cell compartments.[57,58]

The development of polymers with attached proteins or peptide endgroups is fast evolving and a range of conjugation chemistry has been developed. Polymer chains prepared with numerous different polymerization techniques are typically attached to the proteins/peptides using endfunctionalities such as aldehydes, maleimides, pyridyl disulfide, click-chemistry and *N*-hydroxysulfosuccinimide.[59]

Glycopolymers. Incorporation of carbohydrate moieties as either pendant or terminal groups to a polymer chain leads to the effective synthesis of glycopolymer materials.[60,61] These synthetic materials are generally water-soluble, highly polar, biocompatible and occasionally pharmacologically active.[62]

Similar to protein and peptides, carbohydrates can display targeting properties. The asialoglycoprotein receptor (ASGPR) is a transmembrane glycoprotein. It mediates binding, internalization and degradation of compounds such as extracellular glycoproteins that have exposed terminal galactose residues. Consequently, the liver can be targeted using galactose decorated polymer particles.[63–66] A similar activity is expected when incorporating lactose molecules, which consist of β-D-galactose and D-glucose.[67,68]

Con A, a tetrameric lectin known to have a binding affinity for mannose moieties, has been shown to be an activator for cellular signalling events, such as cell adhesion, proliferation, and survival.[69] Several studies have been performed on the binding interactions between Con A and polymers[70] containing mannopyranoside repeat units.

Various polymerization techniques have been developed to synthesize glycopolymers, such as living ionic polymerization, ring opening polymerization, ring opening metathesis polymerization, click chemistry, cyanoxyl-mediated free radical polymerization, ATRP and NMP. These techniques have been reviewed extensively elsewhere.[71]

Crosslinked micelles

Despite the high thermodynamic and kinetic stability of polymeric micelles, further stabilisation can be necessary to avoid disintegration of the aggregate at low concentrations or upon environmental changes such as elevated temperature, altered pH values or increased ionic strength. An easy and promising approach to target a more robust delivery system consists in crosslinking of micelles to stabilize aggregates.

A range of pathways have been reported to achieve further stabilization of self-aggregates. The introduction of reactive or polymerizable endgroups to the hydrophobic block of an amphiphilic block copolymer enables the fixation of the micelle within the micelle core (Fig. 3A).[72,73] Furthermore, the random distribution of functional groups along the hydrophobic block promotes the stabilization of the structure (Fig. 3B).[74] However, this approach limits the loading capacity and affects the drug release. The formation of a triblock copolymer with subsequent crosslinking of the middle block resulted in cosslinking along the interface between hydrophobic and hydrophilic blocks (Fig. 3E).[75] Shell cross-linked micelles, so-called knedels,[76–79] allow the stabilization of the micelle without affecting the loading capacity in the core (Fig. 3C).[80,81] The crosslinking chemistry ranges from carbodiimide and glutaraldehyde to click chemistry and has been reviewed elsewhere.[82] Great potential can be expected by decoration of these crosslinked nanoparticles with reactive moieties.[80]

A slightly different approach can be employed when using charged block copolymers. Negative or positive charges distributed along either block can form strong complexes with polyelectrolytes of the opposite charge. Depending on the environment such as the ionic strength, these polyion complexes can either be very stable or the crosslinking can be reversed (Fig. 3D).[82]

Design of the core-forming block

An important decision when creating micelles for drug delivery purposes concerns the design of the core forming polymers. Only a high compatibility between drug and polymer can ensure high loading capacity.[83] Therefore, each drug delivery system needs to be tailored toward the specific properties of the drug. A range of techniques such as DSC, FT-IR or NMR can measure the compatibility, but also theoretical approaches such as the calculation of partial solubility parameters can be a successful tool to predict the optimum core forming block. Theoretical calculations in combination with appropriate experiments were performed on ellipticine, a cancer drug, and a range of different hydrophobic polymers. The loading capacity could indeed be predicted using solubility parameter. Polymer and drug with similar polarities leads typically to the highest loading. This was especially demonstrated when using a block copolymer based on doxorubicin building blocks to encapsulate a high amount of doxorubicin. While the doxorubicin that was attached as pendant group to the polymer was inactive, it nevertheless allowed a high drug loading due to similarities between block structure and drug.[84] However, a high loading capacity does not always mean that the drug is released at a reasonable rate. Careful fine-tuning is

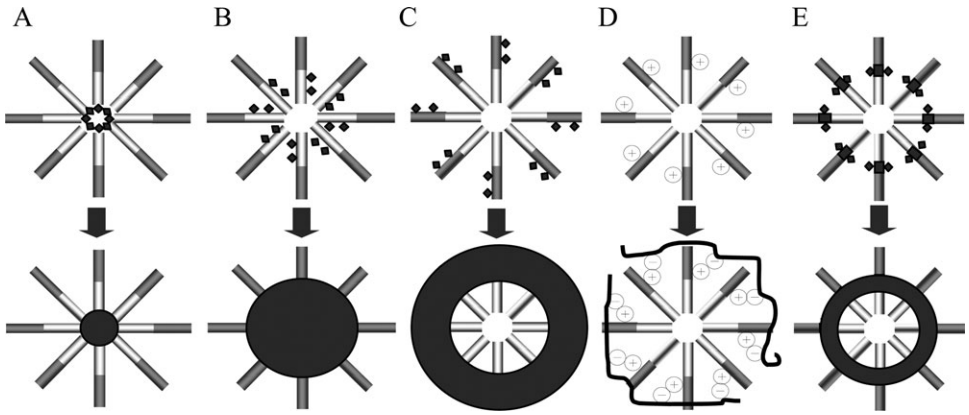

Fig. 3 Crosslinking of micelles *via* functional groups at the end of hydrophobic chain (innercore crosslinked, A), functional groups along hydrophobic chain (core crosslinked, B), functional groups along hydrophilic chain (shell crosslinked, C), polyelectrolyte complex formation (D), functional group along middle block of triblock (interface crosslinking, E).

often required to obtain an optimum between drug loading and rate of release.[83]

Polymeric micelles are generally investigated to encapsulate hydrophobic drugs to increase their solubility in an aqueous environment. However, the delivery of hydrophilic drugs using a polymer matrix is equally of interest to either protect the drug or to increase the circulation time. In order to achieve high loading the drug often needs to strongly interact with the core forming block *via* stronger interaction than van der Waals forces. Examples are the use of cationic polymers to bind negatively charged genes or oligonucleotides[85,86] or the use of complex forming ligands to bind metal containing drugs such as platinum drugs.[87]

RAFT polymerization as a tool for functional polymeric micelles

Looking at the background literature there is a substantial need for novel amphiphilic block copolymers. Especially, block copolymers with functional and reactive groups are in high demand. It is evident that the size and nature of the block copolymer can influence the size, stability, the performance and other parameter of a drug carrier. Versatile tools to carefully fine-tune the block copolymer are essential. Controlled/radical polymerization techniques such as NMP, ATRP or RAFT open up an infinite array of possible structures. RAFT (reversible addition fragmentation chain transfer) polymerization is observed to be a robust technique suitable for different reaction media such as bulk, emulsion, water and suspension. Control is achieved using thiocarbonyl thio compounds. Depending on the structure of the so-called RAFT agent the controlled polymerization of a wide range of monomers can be achieved[88] In addition, easy access to a variety of polymer architectures[89] including block copolymers is possible.

Synthesis of polymers *via* RAFT

The RAFT process deviates from ATRP or NMP by its close relationship to conventional free radical polymerization. Initiated with similar techniques as free radical polymerization (such as thermal, UV, gamma initiation) the process is controlled by an added RAFT agent, a thiocarbonylthio compound (Fig. 4). A broad variety of structures can act as RAFT agents. Depending on the so-called *Z* group (the group adjacent to the carbon of the thiocarbonyl group) and the *R* group (leaving group, which will detach from the thiocarbonyl thio group) the RAFT agents can only sufficiently control the polymerization of one type of monomer (Fig. 4). The structure of the RAFT agent will strongly determine the success of the RAFT process with Z-groups such as –OR or $-NR_2$ being more compatible with the polymerization of reactive radicals such as vinyl acetate, while benzyl groups have a rather stabilising effect on the intermediate radical, thus controlling the polymerization of styrene and methacrylates (Fig. 4). The colourful RAFT agents (from yellow to purple) is responsible for the slight coloration of the final polymer.

Upon initiation of the polymerization with a radical species, the generated macroradical should react instantaneously with the RAFT agent (addition) resulting in a semi-stable radical intermediate that subsequently fragments into the radical leaving group R and the so-called macroRAFT agent (a polymer with thiocarbonylthio group), a polymer with

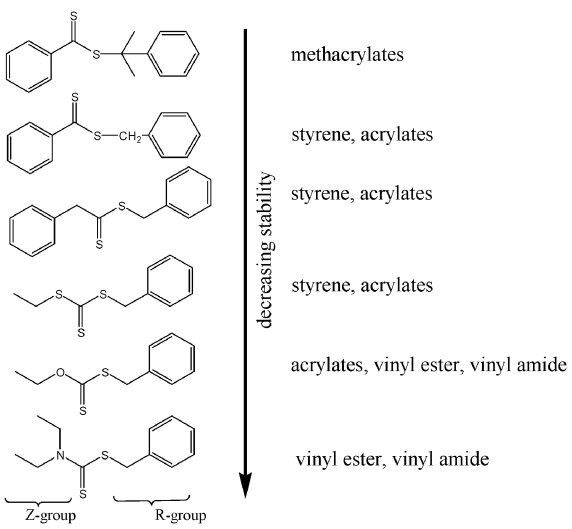

Fig. 4 Variety of RAFT agents with approximate stability of radical intermediate and type of monomer to be used.

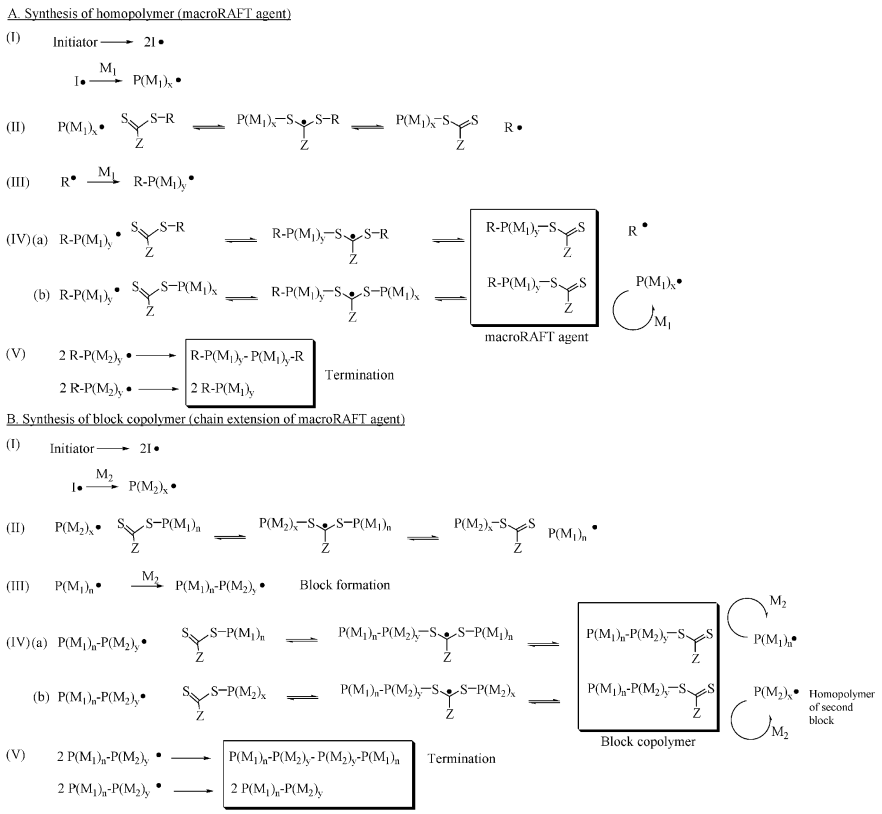

Fig. 5 Synthesis of block copolymers *via* RAFT process.

thiocarbonyl thio endgroup (Fig. 5A, II). The R group will reinitiated the polymerization until further chain addition fragmentation with either the RAFT agent or the macroRAFT agent takes place (Fig. 5A, IV). The resulting polymer obtained from the RAFT process is therefore endfunctionalized with thiocarbonylthio groups. Termination reactions *via* combination or disproportionation are present but significantly suppressed. Occurrence of termination reactions lead to polymer without RAFT terminated chains, thus, dead polymer. The amount of dead polymer is strongly correlated to the amount of radicals in the system and a ratio of RAFT agent to radical initiator concentration of 3 to 10 is usually recommended.

The living character of the polymerization is evident by the linear relationship between molecular weight and conversion according to M_n = [M]/[RAFT] × conversion × M_{Monomer} + M_{RAFT} (where [M] and [RAFT] are the monomer and RAFT agent concentrations, respectively and M_{Monomer} and M_{RAFT} are the molecular weights). Deviations from this linear correlation point usually to insufficient rates of addition to the RAFT agent or to excessive termination reactions.

Once potential side reactions are suppressed, well-defined homopolymers with thiocarbonyl thio endgroups are generated. Block copolymer can then be generated using a range of approaches as discussed below.

Synthesis of block copolymers *via* chain extension

The key to the successful synthesis of block copolymers lies in the presence of the RAFT endgroup of the final product. The polymerization can be restarted in the presence of a new monomer while employing a macroRAFT agent instead of a low molecular weight RAFT agent as a controlling moiety (Fig. 6).

However, the process is not as straight forward as depicted in Fig. 6. The detailed process as seen in Fig. 5 shows the initiation of the second monomer followed by addition and fragmentation with the macroRAFT agent, which has been generated in the initial homo polymerization (Fig. 5B, II). Only the following step, where the polymeric leaving group $P(M_1)^{\bullet}$ polymerizes with the second monomer M_2, results in the generation of block copolymer structures (Fig. 5B, III). The block-like macroradical can then react with either the original macroRAFT agent (IVa) or the macroRAFT agent based on M_2 generated in step II (IVb). It should be noted that step IVb does only result in M_2 macroradicals, which can only form homopolymers based on the second monomer. It becomes clear that the synthesis of block copolymers *via* RAFT always generates homopolymers as side-products. Again, the amount of these impurities as well as the amount of termination reactions leading to multi-block copolymers (*via* combination) can be directly influenced by the amount of radicals born during the process.

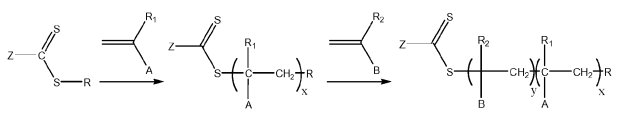

Fig. 6 Synthesis of block copolymers *via* chain extension of a macroRAFT agent.

It is evident that the radical concentration is a major player in the synthesis of well-defined block copolymers assuming that the RAFT agent employed has optimum addition–fragmentation rate constants. The synthesis of the homopolymer, which is the first block of the block copolymer, can possibly be affected by termination reactions (Fig. 5A). This dead polymer $P(M_1)$ will then contaminate the subsequent block copolymer synthesis. The block copolymerization can then theoretically generate homopolymer $P(M_2)$ with RAFT endgroup and dead di- or multiblock copolymer. The final product can thus consist of the block copolymer with RAFT endfunctionality as well as dead homopolymer $P(M_1)$, dead polymer with block structures as well as $P(M_2)$ macroRAFT agent. These side-products can become visible in size exclusion chromatography studies revealing low-molecular weight tailing or high molecular weight shoulders.[90,91] Interestingly, despite possible side-reactions, the experiment usually shows the presence of well-defined products with narrow molecular weight distribution and a molecular weight of the block copolymer increasing linearly with conversion according to M_n = [M]/[RAFT] × conversion × M_{Monomer} + $M_{\mathrm{macroRAFT}}$ (where [M] and [RAFT] are the concentrations of monomer M_2 and macroRAFT agent, respectively and M_{Monomer} and $M_{\mathrm{macroRAFT}}$ are the molecular weights). The above mentioned side-products can be successfully suppressed in many synthesis attempts by careful choice of concentrations (especially the ratio between thiocarbonyl thio groups and the radical initiator) and by opting for a RAFT agent with suitable Z and R-groups.[92] Even if these side-reactions cannot be concealed it has been shown that the product can easily be purified and homopolymer removed. The result is a narrow molecular weight distribution without any low-molecular weight products.[93,94]

Despite all effort to avoid side-reactions during the polymerization, which are derived from the nature of the RAFT process, it should not be forgotten that the RAFT group is a sensitive group that can be destroyed by heat,[95] light,[96] certain pH values[97–99] and solvents that are known to contain oxidizing species such as dioxane or tetrahydrofuran.[100,101] This can potentially lead to the destruction of the thiocarbonylthio endgroup during the polymerization, during storage or during purification *via* precipitation or dialysis. Colour changes are an obvious indication for the depletion of the endgroup.[102] UV-Vis spectroscopic investigation can therefore be used to quantify the amount of active end groups.[103]

The initial decision making process when preparing block copolymers is the sequence in which the polymer is prepared. While many block copolymers can be generated synthesizing either block first some limitations have to be considered. Key is the intermediate radical as depicted in Fig. 5B, II. The ability of the polymeric leaving group to fragment into a macroradical and restart the polymerization—here with the monomer forming the second block—is vital for the successful formation of block copolymers. Not every leaving group can undergo fragmentation at the desired rate. If the second monomer forms a relatively stable radical—such as in methacrylates—the fragmentation will not be directed towards the first block if the macroradical of the first block is less stable. Recommendations on the preparation of the first blocks have been established already in the first publication on block copolymers *via* RAFT.[104] Methacrylyl radicals have a greater leaving capability than styryl or acrylyl radical. It is therefore suggested to prepare a methacrylate-type macroRAFT agent first followed by chain extension with styrene or acrylate-type monomers.

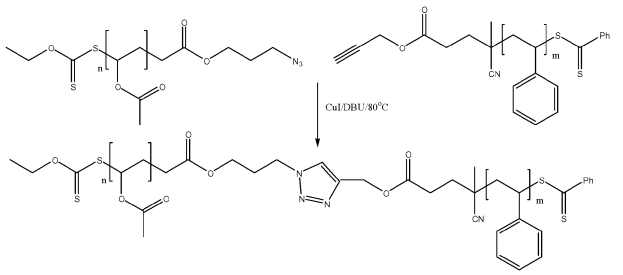

Fig. 7 Synthesis of polystyrene-block-poly(vinyl acetate) *via* combined RAFT and click chemistry.[109]

A range of monomers have been successfully employed to prepare block copolymers. However, limitations of the RAFT process lies in its unsuitability to prepare block copolymers from monomers with disparate reactivities. As mentioned above, groups of monomers can only be polymerized using certain RAFT agents. While the styrene polymerization is living in the presence of dithioesters or trithiocabonates,[105] vinyl acetates can only be controlled using xanthates or carbamides.[106] The quest for a universal RAFT agent is obvious and significant steps forward have been achieved by using the so-called F-RAFT agent with a fluoride group as the Z-group.[107] Alternatively, the two blocks can be married using 'click'-chemistry.[108] In order to generate novel block copolymers *via* RAFT, a xanthate based RAFT agent with azide functionality and a dithioester with alkyne endgroup were employed. The resulting poly(vinyl acetate)-N_3 homopolymer was clicked together with polystyrene–C≡CH in the presence of a copper catalyst resulting in poly(vinyl acetate)-block-polystyrene in high conversions (Fig. 7).[109]

Block copolymers *via* RAFT polymerization and other polymerization techniques

A very elegant way to combine different types of polymer is by attaching a RAFT agent to a polymer made by other techniques. The RAFT agent can be attached to functional polymers either using the Z-group or the R-group. The implications of the type of attachment on the RAFT process is discussed in detail elsewhere.[89] The combination of RAFT made polymers with polycondensates such as polyesters can lead to novel block copolymers. Several synthetic approaches are described in literature.

(a) RAFT agent covalently bound to functional non-RAFT polymer. The RAFT agent has been attached to endfunctional polymers typically *via* ester linkage. The resulting polymeric RAFT agent is then employed in the subsequent RAFT polymerization. The polymers range from poly(ethyleneoxide),[104,110–117] (Fig. 8) kraton,[118] polyethylene[119,120] to poly(dimethylsiloxane)[121]

(b) Functional RAFT agent as an initiator for non-RAFT polymerization. RAFT agents carrying hydroxyl groups were

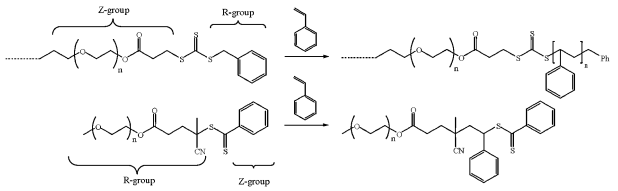

Fig. 8 Synthesis of poly(ethylene oxide)-block-polystyrene *via* Z-group (top)[115] and R-group approach (bottom).[104]

successfully employed in the ring-opening polymerization to generate poly(lactate) endfunctionalized with a RAFT agent (Fig. 9).[122,123] Ring-opening metathesis polymerization of 1,5-cyclobutadiene was proven successful to obtain poly(butadiene)-based block copolymers.[124]

(c) Simultaneous RAFT and ring-opening polymerization. Both polymerizations can be carried out concurrently as it has been demonstrated in the block copolymer synthesis of styrene and poly(ε-caprolactone) using a functional RAFT agent.[125]

(d) Reaction of two polymers. A poly(ε-caprolactone) polymer with diene endfunctionality has been connected *via* hetero Diels Alder cycloaddition (similar to click chemistry) to polystyrene synthesized using pyridyl or phosphoryl containing RAFT agents (Fig. 10).[126]

Amphiphilic block copolymers *via* RAFT for biomedical applications

The tool box available to synthesise block copolymers is highly versatile, allowing the generation of novel block copolymers not only for the preparation of polymeric micelles, but also for other applications. A significant advantage of RAFT polymerization is its robustness to the presence of functional groups. A range of hydrophilic monomers including ones with carboxylate groups or ionic groups can be polymerized without the recourse to protective chemistry. Care should only be taken with basic groups such as amines, which can potentially hydrolyse the RAFT functionality.

From a theoretical point of view the range of potential structures is limitless, once above mentioned parameters such as concentrations and the order of block synthesis has been considered. However, when synthesizing amphiphilic structures the right choice of solvent can be a major obstacle. In order to prepare well-controlled structures it is advised to employ a solvent that can dissolve the macroRAFT agent as well as the monomer of the second block and the final block copolymer. While this problem can be successfully addressed with a range of a suitable solvent that can dissolve hydrophilic as well as hydrophobic structures, there are still several block copolymer structures where a common solvent is absent. This problem can be circumvented by using protection chemistry to

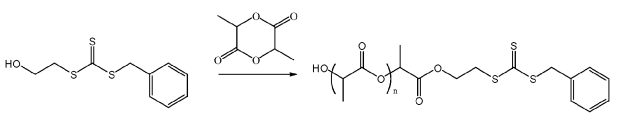

Fig. 9 Synthesis of poly(lactic acid) based RAFT agent.[122,123]

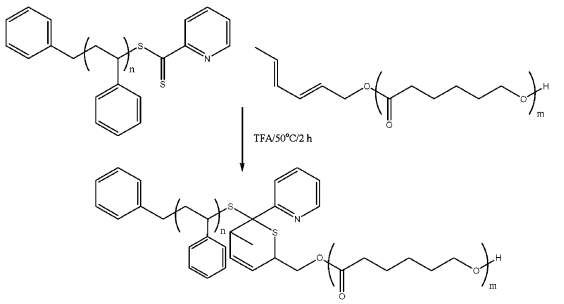

Fig. 10 Hetero-Diels Alder reaction with RAFT agent.

alter the polarity of the monomer. Subsequent deprotection results in the formation of well-defined amphiphilic structures. Acetal protected acrylic acid allowed the synthesis of poly(methyl methacrylate)-*b*-poly(acrylic acid) in solvents such as toluene[127] while amphiphilic block copolymers based on acrylic acid such as polystyrene-*b*-polyacrylic acid is typically polymerized in polar solvents such as *N*,*N*-dimethyl acetamide.[128] Polymers with cationic ammonium side-chains adjacent to a block of polystyrene were prepared by post functionalization to avoid having to work in a heterogeneous polymerization system since no common solvent is known for this structure.[129,130] However, even heterogeneous systems allow the successful synthesis of amphiphilic block copolymers *via* RAFT such as reaction medias where the macroRAFT agent is only dispersed.[131,132] Excellent solubility of all components can, in contrast, not always ensure the formation of well-defined structures. Chain length dependent effects may prevent the requisite instantaneous chain transfer, thus resulting in bimodal molecular weight distributions as it has been demonstrated in the synthesis of polystyrene-*b*-poly(*N*,*N*-dimethacrylamide).[133]

Substantial work has been done regarding the synthesis of block copolymers using the RAFT process. All three techniques—chain extension, using a polymeric (non-RAFT polymer) RAFT agent or combination of two blocks (*i.e.* click)—have been successfully employed to obtain block copolymers. A review summarizing the vast amount of block copolymers obtained *via* RAFT polymerization can be found elsewhere.[134] In the following, polymers are highlighted which are of interest for biomedical applications such as drug delivery.

Water soluble polymers

Neutral polymers. The gold standard of drug delivery—PEO—has widely been utilized in RAFT polymerization. Two approaches were reported: firstly, the conversion of polyethylene glycol into a polymeric RAFT agent by covalent attachment of a RAFT agent to PEO and secondly, the homo- and copolymerization of poly(ethylene glycol) (meth)acrylate (Fig. 11) *via* the RAFT process. The first procedure has been employed to prepare block copolymers with polyethylene glycol blocks in combination with blocks based on styrene,[104] benzylmethacrylate,[104] *N*-vinyl formamide,[110] butyl acrylate,[111] 1,1,2,2-tetrahydrofluorodecyl acrylates,[112] *N*-isopropyl acrylamide,[113] *N*,*N*-dimethyl acrylamide,[114] *N*-acryloxysuccinimide,[114] vinyl acetate and vinyl pyrrolidone,[116] styrene/

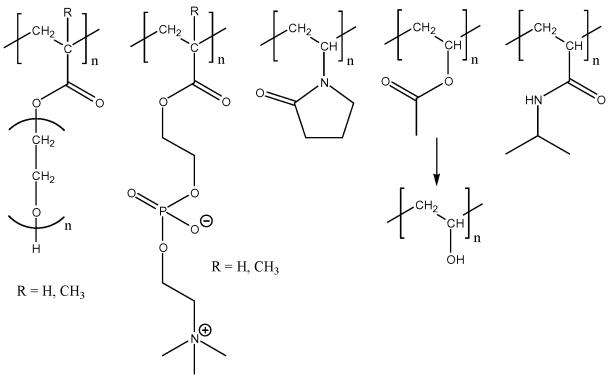

Fig. 11 Examples of water soluble polymers (from left to right: poly(poly ethylene glycol) (meth)acrylate, poly(2-(meth)acryloyloxyethyl phosphorylcholine), poly(*N*-vinyl pyrrolidone), poly(vinyl acetate) and poly(vinyl alcohol), poly(*N*-isopropyl acrylamide)).

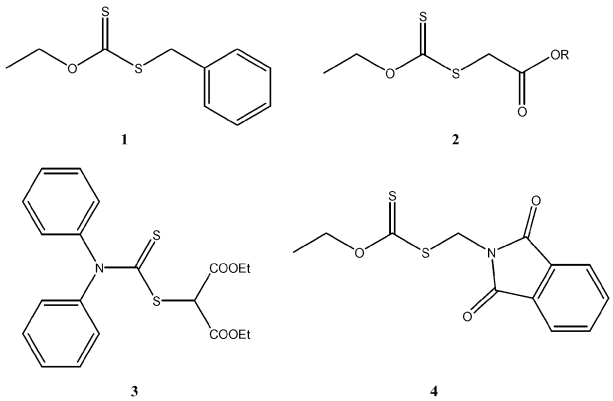

Fig. 12 Selection of RAFT/MADIX agents suitable for the polymerization of *N*-vinyl pyrrolidon or vinyl ester (**1**,[140,142] **2**,[145] **3**,[143] **4**,[144]).

methacrylate[117] and styrene/2-hydroxy ethyl methacrylate.[115] This approach has been described in the section above. Alternatively to poly(ethylene glycol), similar biocompatible properties are expected by preparing poly(poly ethylene glycol) acrylate or methacrylate as a water soluble block (Fig. 11). Both monomers were employed in the synthesis of block copolymers either as the first block and then chain extended for example with zwitterionic monomers[135] or butyl acrylate[135] or they were employed in the chain extension of macroRAFT agents based on *N*,*N*-dimethylamino ethyl methacrylate,[136] *N*,*N*,*N*-trimethylammonium ethyl methacrylate,[138] butyl acrylate[137,138] or glycidyl methacrylate.[139]

PEG based block copolymers prepared *via* RAFT polymerization have been studied regarding their self-assembly. A micellar system based on PEG and poly(*N*,*N*,*N*-trimethylammonium ethyl methacrylate) was investigated concerning its ability to encapsulate fatty acid salts.[138] Another system studied was block copolymers with poly(butyl acrylate) PBA blocks, which resulted in dynamic polymeric micelles with the size of the micelle increasing with the length of the hydrophobic PBA block.[137]

While most examples in the literature focus on PEG as a non-ionic water soluble block, RAFT polymerization opens up avenues to new possible structures. Poly(vinyl pyrrolidone) PVP (Fig. 11) has only briefly been mentioned as a potential hydrophilic block. One of the reason may be the difficult access to well defined structures based on PVP. However, a range of RAFT agents allow now the easy preparation of complex PVP structures such as block copolymers and stars.[140] RAFT agents such as dithiocarbamates or MADIX agents (agents based on xanthates†) are proven to be very effective in the polymerization of vinyl pyrrolidone due to their involvement of the free electron pair of N or O, which lowers the activity of the C=S double bond.[141] Depending on the design of the RAFT/MADIX agent (Fig. 12) the polymerization of vinyl pyrrolidone can proceed in a living fashion.[142–144]

Poly(vinyl alcohol) PVA, the hydrolysis product of poly(vinyl acetate) (Fig. 11), is investigated as highly biocompatible polymer for a range of biomedical applications including certain types of drug delivery. However, no reports have emerged so far using poly(vinyl alcohol) as hydrophilic block in order to prepare polymeric micelles. Similar to PVP, reason may be the absence of suitable polymerization techniques to obtain block copolymers. With the emergence of the RAFT process, vinyl acetate can now be polymerized using similar RAFT/MADIX agents to vinyl pyrrolidone. Well-defined structures including block and starpolymers have been reported.[145–150] However, care has to be taken with impurities. Even small amounts—possibility introduced with the RAFT agent—might inhibit the early stage of the polymerization leading to inhibition periods of several hours.[151]

Both, PVP and PVA have not been investigated yet as hydrophilic polymers for polymeric micelles. However, the facile synthetic approach *via* RAFT will open a window of opportunity to access block copolymer using these highly biocompatible polymers.

Stimuli-responsive polymers. Poly(*N*-isopropyl acrylamide) PNIPAAm (Fig. 11) has widely been mentioned as a thermoresponsive polymer sequence that can potentially be employed whenever an area such as tumours display increased temperatures. There is a vast amount of literature available on the synthesis of PNIPAAm *via* RAFT polymerization including reports on block copolymers of PNIPAAm with *N*,*N*-dimethyl acetamide,[152,153,] methacrylic acid,[154] *N*-acryloylpyrrolidone,[138] methyl methacrylate,[155] benzyl methacrylate,[156] styrene,[157] *tert*-butyl methacrylate,[157] 4-vinylbenzoic acid,[158] *N*-acryloyl glucoseamine,[159] acrylic acid,[160], *N*,*N*-diethylamino ethyl methacrylate,[161] *N*,*N*-dimethylamino ethyl methacrylate[162] and ionic monomers such as *N*-(3-aminopropyl)methacrylamide hydrochloride[163] and sodium 2-(acrylamido)-2-methylpropanesulfonate.[164] The RAFT polymerization of NIPAAm is very robust and can be carried out under a range of temperatures and conditions employing a variety of RAFT agents from dithiobenzoates to trithiocarbonates. RAFT polymerization of NIPAAm is rarely affected by impurities. However, the proof of the living behaviour of the polymerization as evidenced by the linear correlation between molecular weight and monomer conversion is hampered. The exact determination of the molecular weight *via* size exclusion chromatography SEC is difficult and a lot of controversial discussion about the SEC analysis of PNIPAAm can be found in the literature.[32]

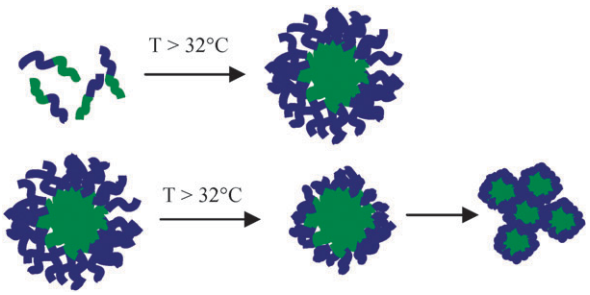

Fig. 13 Aggregation behaviour of PNIPAAm containing block copolymers in water with PNIPAAm in combination with a hydrophilic block (top) and a hydrophobic block (bottom).

Block copolymers based on PNIPAAm and another block can undergo varying changes in aggregation behaviour around the lower critical solution temperature (LCST). In combination with a hydrophobic block, water soluble aggregates such as micelles are formed below the LCST while the polymers becomes fully hydrophobic above 32 °C, thus the polymer precipitates or collapses. A fully water soluble structures below 32 °C is formed when the PNIPAAm block is combined with an hydrophilic block. With heating above the LCST, the formation of micelles or other aggregates is observed (Fig. 13).

Both types of block copolymer using NIPAAm were generated using the RAFT process.

Thermoresponsive hybrid nanoparticles with a silica core were obtained by the sol-gel process using poly(*N*-isopropyl acrylamide)-*b*-poly(γ-methacryloxypropyltrimethoxy silane). The core-shell particles with a crosslinked core display a two-stage collapse upon heating.[165]

The size of the hydrophobic block can have an influence of the LCST of PNIPAAm as demonstrated using either polystyrene or poly(methylmethacrylate) as hydrophobic blocks.[157] If the hydrophilic block was short compared to the hydrophobic block large aggregates were formed instead of micelles. While micelles compress slowly the PNIPAAm corona, the PNIPAAm shell collapses suddenly in large aggregates.

PNIPAAm, in combination with hydrophilic blocks such as poly(*N*,*N*-dimethyl acetamide)[166,167] or poly(acryloyl glucosamine),[168] results in the reversible association of this block copolymer above the LCST.

Double responsive micelles were prepared from PNIPAAm in combination with 3-[*N*-(3-methacrylamidopropyl)-*N*,*N*-dimethyl]ammoniopropane sulfonate (SPP). While PNIPAAm displays a lower critical solution temperature (LCST) at 32 °C, PSSP has an upper critical solution temperature at 12.5 °C. The block copolymers are therefore only fully soluble in a small temperature window while they form aggregates or inverse aggregates depending on the temperature.[169]

It is desirable for applications such as drug delivery to finetune the transition of the PNIPAAm solubility to accommodate temperatures in physiological environments. An increased LCST was achieved by random copolymerization of PNIPAAm with *N*,*N*-dimethyl acetamide. The micelles obtained with poly(benzyl methacrylate) as a hydrophobic block collapsed at values of around 40 °C, which was additionally influenced by the RAFT endfunctionality or other endgroups.[170] In general, the influence of the endgroup of PNIPAAm on the behaviour is not to be underestimated and can have a significant influence on the aggregation behaviour.[171]

Other polymers. The list of potential polymers synthesized *via* RAFT polymerization for drug delivery purposes is endless. Micelles based on phosphorylcholine[172,173] were successfully prepared. The amphiphilic block copolymer poly-(2-methacryloyloxyethylphosphorylcholine)-*b*-poly(butyl methacrylate) was investigated for its ability for form a micellar carrier for paclitaxel.[172] Worth mentioning here also are cationic polymers, which are investigated as carrier of genes and oligonucleotides due to their electrostatic interaction with negatively charged groups. Poly(dimethyl amino ethyl) methacrylate was synthesized *via* RAFT polymerization and employed as gene delivery carrier.[136,174] The list of potential water soluble monomers is extensive and is summarized in a detailed review elsewhere.[175]

Glycopolymers. The incorporation of carbohydrate moieties has been shown not only to improve biocompatibility, but also to add bioactive features to the drug carrier. The synthesis of well-defined sugar-containing polymers, so-called glycopolymers, demands frequently the utilization of protected monomers. The polymerization step is therefore followed by a deprotection step. RAFT polymerization, in contrast, does not require the recourse to protecting chemistry. The polymerization can therefore be carried out in water as demonstrated for the first time using methacryloxyethyl glucoside (Fig. 14).[176] Further polymerizations of unprotected glycomonomers in water or protic solvents followed using a range of sugars.[177–184] However, some monomers were still polymerized *via* RAFT polymerization in their protected state probably because of the bigger selection of available solvents, which also ensures a better solubility of many RAFT agents.[185–189] (Fig. 14)

Block copolymers suitable for the preparation of micelles with glycopolymer corona were prepared by chain extension of glycopolymer macroRAFT agent with 2-(*N*,*N*-dimethylamino) ethyl methacrylate resulting in block copolymers with narrow molecular weight distribution carrying a pH responsive block.[185]

Chain extending poly(acryloyl glucosamine) with NIPAAm result in thermo-responsive micelles, which disintegrate into unimers below the LCST.[168] The unimer–micelle transition was observed to be at similar temperatures as the LSCT of PNIPAAm homopolymer indicating a good phase separation of both blocks. In order to vary the thermo-responsive behaviour of PNIPAAm, glycomonomers were randomly copolymerized with NIPAAm. The resulting polymers showed an increased LCST with increasing amount of randomly distributed glycomonomers. In addition, the LCST could be tailored by the spacer chain length of the glycomonomer.[189]

Block copolymers based on a glycopolymer block and polystyrene or poly(methyl methacrylate) showed aggregate formation and a high stability against disintegration as determined *via* CMC measurements.[186]

The work on glycopolymers *via* RAFT has been extended beyond more traditional block copolymers using click chemistry. Block copolymers based on poly(vinyl acetate) and

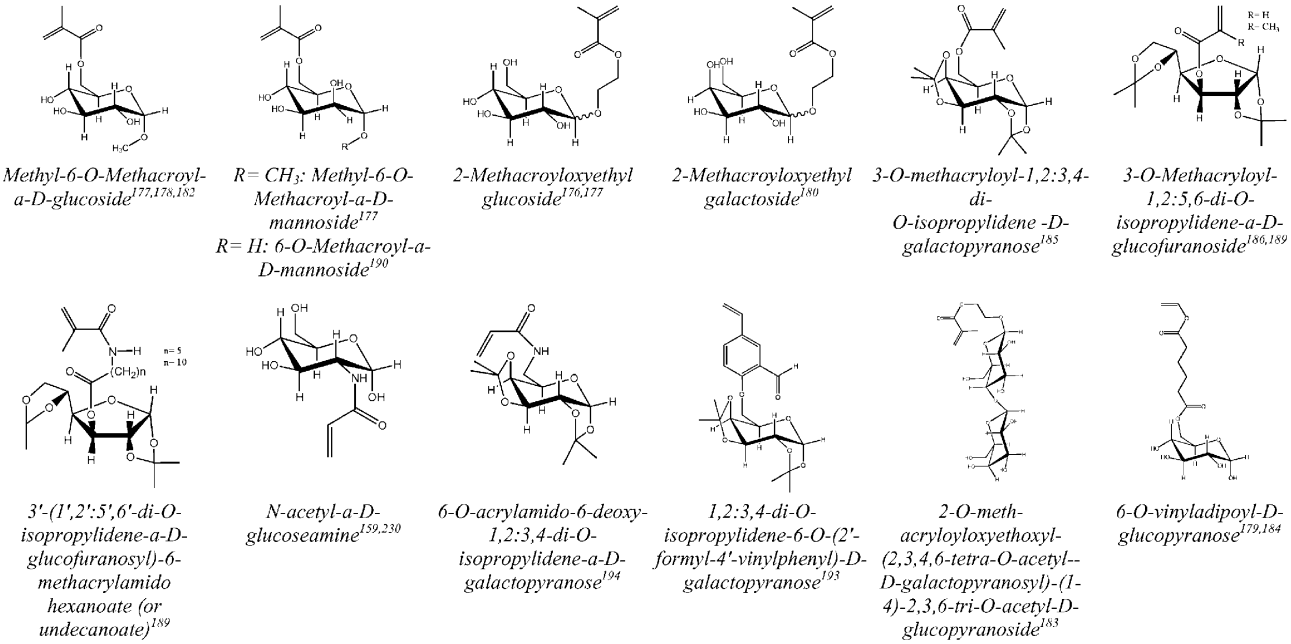

Fig. 14 Glycomonomers polymerized using the RAFT process.

poly(6-*O*-methacryloyl mannose) were obtained *via* combination of blocks *via* click functionalities. The resulting block copolymer self-assembled in aqueous solution.[187]

When designing glycopolymer it has to be considered that depending on the attachment of the sugar to the polymer backbone the bioactivity may be lost. While mannose attached to a polymer backbone *via* 1-position shows a strong binding activity towards concanavalin A, poly(6-*O*-methacryloyl mannose) looses its activity.[190] Computational work suggests that free hydroxyls at the 3-, 4-, *and* 6-carbon positions of mannose dictate the binding ability of Con A.[191]

For many applications it is not necessary to employ a glycopolymer as the water soluble block. A block copolymer with a carbohydrate endgroup can already serve as a precursor for a highly functional micelle. Block copolymers with endfunctional carbohydrate moieties can easily be generated by joining the sugar to the RAFT agent. Depending on the site of attachment to the RAFT agent (R- or Z-group) the sugar will be part of the initiating group or part of the Z-group, hence close to the thiocarbonyl thiogroup. A RAFT agent containing sugar as a part of the R-group was utilized to prepare carbohydrate endfunctionalized poly(*N*-acryloyl morpholine).[192]

Protein/peptide–polymer conjugates. As outlined in an earlier review article the pathways to attach proteins or peptides are manifold ranging from aldehydes, maleimides, biotin, pyridyl disulfide, click-chemistry to *N*-hydroxysulfosuccinimide.[59] These functional groups can be easily incorporated into RAFT agents leading to polymers with reactive endgroups. Depending on the design of the RAFT agent, these functional groups will be located at the end of the initiating group (R-group) or close to the RAFT group (Z-group). Considering the stability of the RAFT group as discussed earlier the site of attachment of the RAFT agent can have implications on the stability of the resulting protein–polymer conjugate (Fig. 15).

Two approaches were described in the literature: the formation of endfunctional polymer with the subsequent conjugation to proteins or the conjugation of a functional RAFT agent to the protein followed by the RAFT polymerization.

A popular way of conjugating (strept)avidin is *via* biotin–streptavidin conjugation. The strong affinity of avidin or its proxy, streptavidin, for biotin with a dissociation constant K_d of 10^{-14}–10^{-15} mol L^{-1}, has been well recognized as one of the strongest non-covalent bonds of proteins and enzymes. RAFT agents with biotin endgroups were employed in the polymerization of *N*-acryloyl morpholine,[192] 6-*O*-acrylamido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose,[194] *N*-isopropyl acrylamide,[195] or in the fomation of poly(hydroxypropyl methacrylate)-*b*-poly(*N*-isopropyl acrylamide) block copolymers with biotin endfunctionality.[196]

RAFT agents with pyridyl disulfide groups were employed in the polymerisation of poly(ethylene glycol)acrylate and butylacrylate. The resulting pyridyl disulfide-terminated block copolymer can quantitatively react with thiol containig compounds such as bovine serum albumin (BSA).[197]

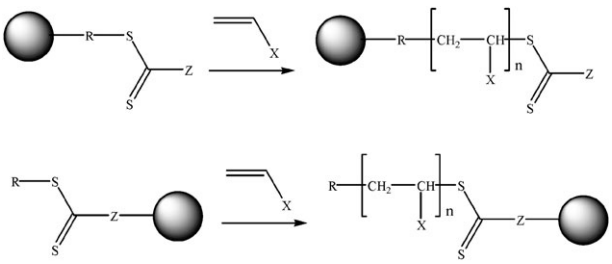

Fig. 15 Design of reactive RAFT agent for protein/peptide attachment with the functional group as a part of the R-group (top) or Z-group (bottom).

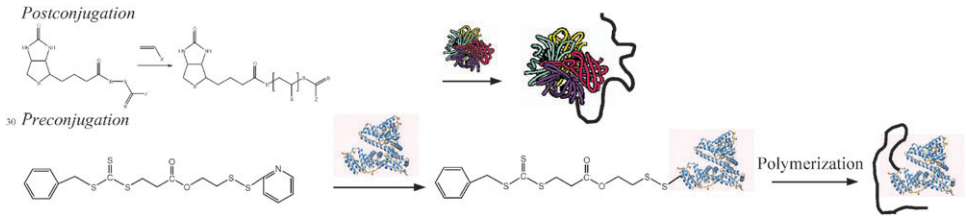

Fig. 16 Synthesis of polymer–protein conjugates *via* RAFT polymerization.

Postmodification of RAFT made polymers *via* $NaBH_4$ generates polymers with thiol functionality (see below postmodification). Peptides or oligonucleotides attached to this terminal thiol lead to conjugates with high biological activity as demonstrated in a molecular recognition assay.[198]

In addition, it has been shown that RAFT polymerization in the presence of RAFT modified proteins can be carried out successfully (Fig. 16). Bovine serum albumin (BSA) was conjugated to a RAFT agent *via* disulfide bridge using a pyridyl disulfide terminated RAFT agent.[199,200] The resulting BSA-RAFT agent was polymerized with poly(ethylene glycol)-acrylate or *N*-isopropyl acrylamide resulting in well-defined polymer protein conjugates without loosing the specific bioactivity of BSA.

Peptides with RAFT endfunctionality were used in the polymerization of *n*-butyl acrylate. The length of the attached polymer chain was found to influence the self-assembly of the peptide–polymer conjugate.[201,202] RAFT agents based on cysteine and glutathione can directly be converted into a RAFT agent by using the thiol group of the aminoacid to form trithiocarbonate.[203] These RAFT agents were found to control the synthesis of various block copolymers in a living manner.

Next to the synthesis of polymer—protein conjugates using reactive RAFT agent, proteins can also be immobilized using block copolymers with one block being composed of monomers, which are reactive towards proteins. *N*-acryloxysuccinimide can readily react with amino groups of proteins/peptides. Therefore, random and block structures of poly(*N*-acryloxysuccinimide) were prepared as a reactive backbone to immobilize proteins.[204–207] Not only proteins were successfully bound to the reactive backbone, but also nucleic acids and plasmid DNA.[206]

A multivalent inhibitor of anthrax toxin was obtained by reacting a particular peptide with a random copolymer based on *N*-acryloxysuccinimide.[208]

A glycomonomer with additional aldehyde functionality as reactive group was polymerized to well defined surface-active polymers and conjugated to BSA.[193]

The bioactive peptide RGD and other peptides/aminoacids were immobilized onto a reactive backbone based on *p*-nitrophenyl methacrylate and diethoxypropyl methacrylate.[209,210]

Aminoacids themselves can be used as a building block in order to prepare synthetic protein-like polymers. L-phenylalanine and L-proline were converted into acrylamides and polymerized into well-defined polymers *via* the RAFT process.[211–213] Poly(L-glutamic acid)-*b*-poly(*N*-isopropyl acrylamide) were synthesized by a combination of ring-opening polymerization of γ-benzyl-L-glutamate *N*-carboxyanhydrides and RAFT polymerization of *N*-isopropylacrylamide.[214]

Other targeting groups. Folate containing block copolymers have been synthesized *via* combination of RAFT and click chemistry to generate particles targeting folate receptors.[215]

Crosslinked micelles. The benefits of crosslinked micelles became evident in earlier studies. Synthesis of block copolymers for further crosslinking *via* RAFT polymerization may have an advantage since the incorporation of functional groups for post reaction is facilitated. The reason is the robustness of the RAFT process against most functional groups. Apart from amines, no other functional groups are known to affect the success of the RAFT process. Even amine containing monomer can be polymerized in a living fashion if either protected or as the hydrochloride salt.[216,217]

The advantage of the RAFT process can be demonstrated when preparing block copolymers for polyion complex micelles (Fig. 3D). Ionic groups are required to lock in the structure by formation of complexes with added polyelectrolytes. Triblock copolymers poly[(*N*,*N*-dimethylacrylamide)-*b*-(*N*-acryloyl alanine)-*b*-(*N*-isopropylacrylamide)] was self-assembled at temperatures above the LCST. Further addition of a polycation fixates the connecting layer between shell and core (poly(*N*-acryloyl alanine)), but can be reversed by changing the ionic strength.[218] The temperature and pH responsiveness of polyelectrolyte interlocked polymers using similar monomers was additionally investigated.[219] This approach can be extended to different charged groups as demonstrated by locking vesicles from poly(*N*-(3-aminopropyl)methacrylamide hydrochloride)-*b*-(*N*-isopropylacrylamide) with poly(*N*-(3-aminopropyl)methacrylamide hydrochloride.[220]

Permanent crosslinking was achieved in a one-pot crosslinking approach using RAFT generated well-defined block copolymers from maleic anhydride and styrene.[221]

Reactive *N*-acryloxysuccinimide was incorporated into the triblock copolymer poly(ethylene oxide)-*b*-poly(*N*,*N*-dimethylacetamide-ran-*N*-acryloxysuccinimide)-*b*-poly(*N*-isopropylacrylamide). Crosslinking with diamines permanently hooked the self-assembled structure.[222] However, it is possible to achieve a less permanent crosslinking. Reversibility upon external stimuli can be introduced when using a disulfide containing diamine. In the presence of thiols, which are present in abundance in peptides and proteins, the disulfide bridge breaks allowing disintegration into unimers.[223] A similar approach to these thiol degradable micelles has been applied using another block copolymer, poly(ethylene oxide)-*b*-poly(*N*-isopropylacrylamide-co-*N*-acryloxysuccinimide).[224]

A very different avenue to crosslink the self-assembled structure can be approached by employing the RAFT process itself. The RAFT endgroup can be utilized to crosslink the

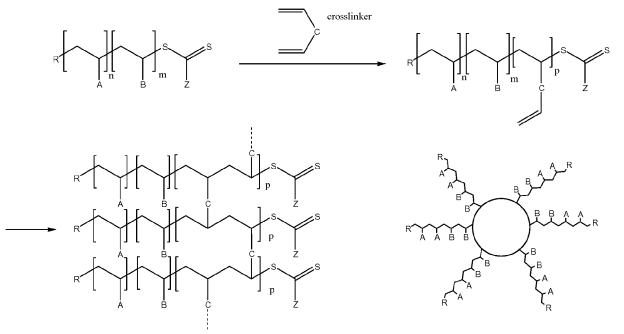

Fig. 17 Schematic drawing to core-crosslinked micelles *via* chain extension of block copolymers with divinyl compounds.

structure by further chain extension to a triblock copolymer. The third block will then be based on a divinyl compound, which will in addition act as a crosslinker. Depending on the position of the RAFT endgroup (located at the end of hydrophilic or hydrophobic block) core or shell crosslinking can be achieved.

Core crosslinked micelles are derived from a technique to obtain star polymers with an arm-first approach. A divinyl compounds, the crosslinker, is added to the polymerization resulting in crosslinking of the participating block copolymers (Fig. 17). In contrast to the star polymer synthesis, a selective solvent is employed that can dissolve the shell, but not the core of the resulting core-shell structure, which facilitates the formation of spherical structures. Two approaches can be pursued. Firstly, the formation of block copolymer followed by self-assembly and triblock formation (crosslinking) in the micelles (Fig. 17) or, secondly, the one-pot approach using a macroRAFT agent, which will be mixed with crosslinker and further monomer resulting in a fully crosslinked, gel-like core.

The one-pot process was successfully applied using PEO macroRAFT agent in a mixture of ethanol–tetrahydrofurane as solvent. Styrene/divinyl benzene was then added to form a fully crosslinked core.[225] Similar approaches using other systems are reported using divinyl benzene or ethylene glycol acrylate as crosslinkers.[226,227]

While this pathway leads to a fully crosslinked core, the self-assembly of block copolymers followed by triblock extension with a divinyl compound does only result in a single point of contact of the block copolymer chains in the centre of the core. The successful synthesis of core-crosslinked micelles was demonstrated using either a poly(2-hydroxy ethyl)-acrylate-*b*-poly(*n*-butyl acrylate)-RAFT[228] or a poly(*N*-isopropyl)-acrylamide-*b*-polystyrene-RAFT[229] block copolymer. Upon self-assembly of the block copolymer in a selective solvent micelles were obtained with the RAFT group being located in the core. Subsequent addition of crosslinking agent results in the fixation of the aggregate.

The permanent fixation of the micelle is, as outlined above, not always desirable and sudden decomposition of the crosslinker can accelerate drug release. Poly(*N*-acryloyl glucoseamine)-*b*-(*N*-isopropyl acrylamide) was crosslinked with the acetal containing divinyl compound 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane.[230] While the micelle was stable at varying temperatures once crosslinked, low pH values resulted

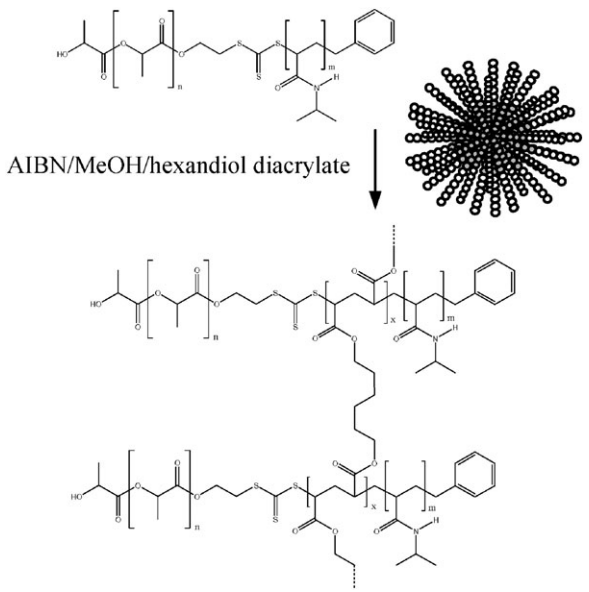

Fig. 18 Crosslinking at the nexus of the block copolymer.[122]

in the formation of unimers due to cleavage of the acetal group. A similar pathway was taken by crosslinking the core of poly(polyethyleneglycol methyl ether methacrylate)-*b*-poly(5′-*O*-methacryloyl uridine) with bis(2-methacryloyloxyethyl)disulfide. While the crosslinked micelle is stable in most aqueous solutions, it rapidly degrades into the underlying block copolymer in the presence of thiols, which rapidly cleaves the disulfide group.[231]

The RAFT endgroup can also be utilized for crosslinking when located at the water soluble block resulting in shell-crosslinking.[136] A Poly(*N*,*N*-dimethylamino ethyl methacrylate) core was surrounded by a poly(polyethyleneglycol methacrylate) shell, which was crosslinked using ethyleneglycol dimethacrylate mixed with further polyethyleneglycol methacrylate. A setback of this approach is, however, the high occurrence of intermicellar crosslinks. The so-stabilized structure was employed as gene delivery carrier. The toxic cationic core was shielded by a biocompatible shell.

Unique to the RAFT process is crosslinking on the nexus between core and shell (Fig. 18). When preparing a macro-RAFT agent using other polymerization techniques such as ring-opening polymerization, the RAFT agent can be located at the connection between both block after the RAFT polymerization. Prerequisite is, however, that the non-RAFT agent is connected *via* the Z-group. Upon self-assembly and addition of divinyl compounds a crosslinked layer at the interface is generated.[122]

Toxicity of RAFT agents and post modification of RAFT endgroups

As highlighted above, functional endgroups can be introduced *via* the RAFT agent. A range of functional groups are reported ranging from carboxyl, amino, metal ligands to fluorescing group. Details can be found in reviews on RAFT polymerizations highlighting the vast variety of available RAFT agents.[88,232] Concern have, however, been raised regarding the suitability of RAFT polymerization for

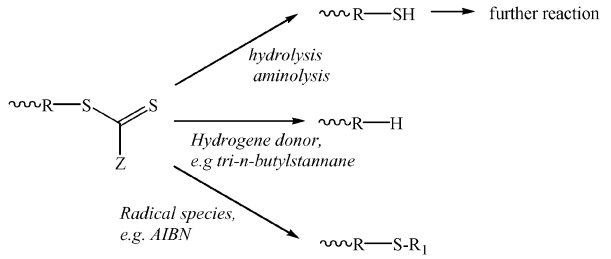

Fig. 19 Endgroup modification of thiocarbonyl thio group.

biomedical application. While some RAFT agents have been shown to be non-toxic[132] other can be quite fatal to an assay of fibroblast cells.[233] Since the RAFT agent is easily subject to hydrolysis nothing is known about the fate and toxicity of degradation products. Despite the validity of these concerns, it has to be considered that the RAFT group can easily be eliminated from the final block copolymer. This is, however, only possible when the RAFT agent does not act as a linker between parts of the RAFT agent. When using functional RAFT agents to create ω-terminated block copolymers the functional group should therefore be located on the R-group.

The RAFT end group removal is often an unwanted side reaction during the polymerization caused by temperature to varying degrees,[234–238] by oxidation[100,239] or by UV-irradiation.[96,240] (It should, however, be noted here that the RAFT endgroup is stable under gamma irradiation[241]). An excess of radicals such as by the addition of a vast amount of azoinitiator can fully remove the RAFT endgroup replacing it with the radical species (Fig. 19).[242] Hydrogen atom donor such as tri-*n*-butylstannane could effectively replace the RAFT endgroup by a hydrogen (Fig. 19).[243] A range of hydrogen donor compounds were investigated in detail.[244]

Hydrolysis and aminolysis using bases such as hydroxides or amines usually results in quantitative endgroup removal depending on pH value and molecular weight.[245] Investigations confirmed the formation of thiol endgroups as a result of the RAFT group cleavage.[246] However, side reactions such as disulfide bridges, eliminations to form vinyl endgroups or the formation of cyclic thiolactones can hamper this approach.[247] Reduction in the presence of sodium bisulfide,[248], $NaBH_4$[249] or Zn–acetic acid[243,250] can only temporarily fix this problem. An elegant approach can be the subsequent reaction of thiols with maleimide[251] or α,β-unsaturated carbonyl derivatives.[252] Thiols endgroups were also modified using iodides in order to obtain α,ω-dicholersteryl or α,ω-dipyrenyl polymers.[253]

Depending on the technique, the RAFT endgroup has been converted into thiol, hydrogene, vinyl groups or others. The similarity of all these technique is the loss of colour, which was derived from the intense UV-vis absorption of the thiocarbonylthio functionality.

Conclusions

Drug delivery using polymeric micelles grew out of its infant state with some block copolymers being currently tested in clinical trials.[254,255] These first encouraging results confirm that block copolymers can indeed benefit drug treatment. Careful tailoring of the hydrophobic and the hydrophilic blocks can further enhance and optimize the drug carrier. It is therefore clear that drug delivery requires a synthetic tool, which is highly robust. The RAFT process was demonstrated to be a versatile tool to obtain functional block copolymers to generate micelles for drug delivery purposes. Especially the ease of incorporation of biological moieties such as sugars, proteins or peptides makes this process highly attractive. The process is stable in the presence of most functional groups and can be employed to control monomers such as vinyl acetate or *N*-vinyl pyrrolidone, which can now potentially take on a role in biomedical applications similar to the "gold standard" polyethylene glycol. Concerns raised regarding the toxicity of the RAFT endgroup are obsolete since the RAFT group can easily be converted. However, despite all advantages of this process, it should not be forgotten that the RAFT process is a radical process with all its side reactions such as termination and chain transfer to other species.

The RAFT process is now in its tenth year of existence[256] and is here to open up roads to before unthinkable structures usually with only a few simple steps. While there are only a few cases where RAFT made polymers have actually been used for drug delivery purposes, this article should encourage the reader to explore the RAFT process as an easy tool to generate novel architectures.

Acknowledgements

MS would like to thank the PhD students and post-doctoral researchers who have so dedicatedly worked on the theory and practice of complex architecture polymers *via* RAFT polymerization in the CAMD laboratories over the past 8 years, including (in no particular order) Hugh Chaffey-Millar, Dr Julien Bernard, Dr Damien Quemener, Dr Xiajuan Hao, Dr Anthony Granville, Dr Maude LeHellay, Dr Cyrille Boyer, Dr Jingquan Liu, Dr Volga Bulmus, Dr Cathy Lefay, Dr Sebastian Sinnwell, Andrew Inglis, Dr Andrew Ah Toy, Kok Hou Wong, Dr Joan Chiu, Maribel Hernández-Guerrero, Ling Zhang, Michelle Hales, Dr Luca Albertin, Tommy Pai, Uyen Nguyen, Anja Goldmann, Lyndal McDowall, Simon Ting and Raymond Joso and many others. I also gratefully acknowledge funding from the *Australian Research Council* (ARC). I like to thank the University of New South Wales UNSW for generous center funding as well as the funding of numerous PhD scholarships. I also like to acknowledge my colleagues in CAMD, Prof. Tom Davis and Istvan Jacenyk (Centre manager) and Dr Leonie Barner (Research manager). I would especially like to thank my colleague Prof. Christopher Barner-Kowollik, who—after 8 years of successful collaboration and office sharing—will leave CAMD to take up a position at the University of Karlsruhe, Germany.

Notes and references

1. K. E. Uhrich, S. M. Cannizzaro, R. S. Langer and K. F. Shakesheff, *Chem. Rev.*, 1999, **99**, 3181.
2. L. W. Seymour, R. Duncan, J. Strohalm and J. Kopecek, *J. Biomed. Mater. Res.*, 1987, **21**, 1341.
3. S. Stolnik, L. Illum and S. S. Davis, *Adv. Drug Delivery Rev.*, 1995, **16**, 195.
4. L. Hongbo, S. Farrell and K. Uhrich, *J. Controlled Release*, 2000, **68**, 167.

5. Y. Matsumura and H. Maeda, *Cancer Res*., 1986, **46**, 6387.
6. K. Letchford and H. Burt, *Europ. J. Pharm. Biopharm*., 2007, **65**, 259.
7. N. Nishuyama, S. Okazaki, H. Cabral, M. Miyamoto, Y. Kato, Y. Sugiyama, K. Nishio, Y. Matsumura and K. Kataoka, *Cancer Res*., 2003, **63**, 8977.
8. G. Riess, *Prog. Polym. Sci*., 2003, **28**, 1107.
9. J.-F. Gohy, *Adv. Polym. Sci*., 2005, **190**, 65.
10. G. S. Kwon, M. Naito, M. Yokoyama, Y. Sakurai and K. Kataoka, *Langmuir*, 1993, **9**, 945.
11. P. Linse, "Modelling of self-assembly of block copolymers in selective solvents", in *"Amphiphilic block copolymers: self-assembly and applications"*, ed. P Alexandridis and B Lindman, Elsevier, Amsterdam, 2000, p. 13.
12. A. Halperin, *Macromolecules*, 1987, **20**, 2943–2946; M. Daoud and J. P. Cotton, *J. Phys. (Fr)*, 1982, **43**, 531.
13. J. Noolandi and K. M. Hong, *Macromolecules*, 1983, **16**, 1443; L. Leibler, H. Orland and J. C. Weeler, *J. Chem. Phys*., 1983, **79**, 3550; R. Nagarajan and K. Ganesh, *Macromolecules*, 1989, **22**, 4312.
14. K. Binder and M. Müller, *Curr. Opin. Colloid Interf. Sci*., 2000, **5**, 315.
15. *"The physics of block copolymers"*, ed. I. W. Hamley, Oxford Science Publications, Oxford, 1998Z. Tuzar, "Overview of polymer micelles", in *NATO ASI series series E: Applied sciences*, ed. S. E. Webber, P Munk and Z Tuzar, Kluwer, Dordrecht, 1996, vol. 327, p. 367.
16. C. O. Rangel-Yagui, A. P. Junior and L. C. Tavares, *J. Pharm. Pharmaceut. Sci*., 2005, **8**, 147.
17. J. R. Leiza and J. M. Asua, "Copolymers", in *Kirk-Othmer Encyclopedia of Chemical Technology*, 5th edn, 2004, vol. 7, pp. 607–669; W. A. Braunecker and K. Matyjaszewski, *Prog. Polym. Sci*., 2007, **32**, 93; T. Yokozawa and A. Yokoyama, *Prog. Polym. Sci*., 2007, **32**, 147; M. Zintl and B. Rieger, *Angew. Chem., Int. Ed*., 2007, **46**, 333; N. Hadjichristidis, M. Pitsikalis and H. Iatrou, *Advances Polym. Sci*., 2005, **189**, 1.
18. M. L. Adamas, A. Lavasanifar and G. S. Kwon, *J. Pharm. Sci*., 2003, **92**, 1343.
19. M. T. Peracchia, C. Vauthier, C. Passirani, P. Couvreur and D. Labarre, *Life Sci*., 1997, **61**, 749.
20. A. Kabanov, E. V. Batrakova and V. Y. Alakhov, *J. Controlled Release*, 2002, **82**, 189.
21. W.-J. Lin, L.-W. Juang and C.-C. Lin, *Pharm. Res*., 2003, **20**, 668; T. Riley, S. Stolnik, C. R. Heald, C. D. Xiong, M. C. Garnett, L. Illum, S. S. Davis, S. C. Purkiss, R. J. Barlow and P. R. Gellert, *Langmuir*, 2001, **17**, 3168.
22. H. S. Yoo and T. G. Park, *J. Controlled Release*, 2001, **70**, 63–70.
23. X. Shuai, T. Merdan, F. Unger, M. Wittmar and T. Kissel, *Macromolecules*, 2003, **36**, 5751; L. Piao, Z. Dai, M. Deng, X. Chen and X. Jing, *Polymer*, 2003, **44**, 2025.
24. S. Zhang, J. Qing, C. Xiong and Y. Peng, *J. Polym. Sci. Part A Polym. Chem*., 2004, **42**, 3527; A. Harada and K. Kataoka, *Macromolecules*, 1995, **28**, 5294; A. Lavasanifar, J. Samuel and G. S. Kwon, *J. Biomed. Mater. Res*., 2000, **52**, 831; A. Lavasanifar, J. Samuel and G. S. Kwon, *Adv. Drug Del. Rev*., 2002, **54**, 169.
25. G. Gaucher, M.-H. Dufresne, V. P. Sant, N. Kang, X. Dusica Maysinger and J.-C. Leroux, *J. Controlled Release*, 2005, **109**, 169.
26. A. Benahmed, M. Ranger and J. C. Leroux, *Pharm. Res*., 2001, **18**, 323; L. Luo, M. Ranger, D. G. Lessard, D. Le Garrec, S. Gori, J. C. Leroux, S. Rimmer and D. Smith, *Macromolecules*, 2004, **37**, 4008.
27. K. Ishihara, N. P. Ziats, B. P. Tierney, N. Nakabayashi and J. Anderson, *J. Biomed. Mater. Res*., 1991, **25**, 1397.
28. Y. Ma, Y. Tang, N. C. Billingham and S. P. Armes, *Biomacromolecules*, 2003, **4**, 864.
29. J. Kost and R. Langer, *Adv. Drug Delivery Rev*., 2001, **46**, 125.
30. C. J. F. Rijcken, O. Soga, W. E. Hennink and C. F. Van Nostrum, *J. Controlled Release*, 2007, **120**, 131.
31. T. E. Dudar and R. K. Jain, *Cancer Res*., 1984, **19**, 605.
32. H. G. Schild, *Prog. Polym. Sci*., 1992, **17**, 163.
33. Y. Zhang, M. Juang, J. Zhao, X. Ren, D. Chen and G. Zhang, *Adv. Funct. Mater*., 2005, **15**, 695; M. D. C. Topp, P. J. Dijkstra, H. Talsma and J. Feijen, *Macromolecules*, 1997, **30**, 8518; P. W. Zhu and D. H. Napper, *Langmuir*, 2000, **16**, 8543; X. P. Qiu and C. Wu, *Macromolecules*, 1997, **30**, 7921; D. Neradovic, O. Soga, C. F. Van Nostrum and W. E. Hennink, *Biomaterials*, 2004, **25**, 2409.
34. G. Martina and R. K. Jain, *Cancer Res*., 1994, **54**, 5670.
35. N. Murthy, J. Campbell, N. Fausto, A. S. Hoffman and P. S. Stayton, *J. Controlled Rel*., 2003, **89**, 365.
36. A. M. Funhoff, S. Monge, R. Teeuwen, G. A. Koning, N. M. E. Schuurmans-Nieuwenbroek, D. J. A. Crommelin, D. M. Haddleton, W. E. Hennink and C. F. van Nostruma, *J. Controlled Release*, 2005, **102**, 711; P. van de Wetering, N. M. E. Schuurmans-Nieuwenbroek, M. J. van Steenbergen, D. J. A. Crommelin and W. E. Hennink, *J. Controlled Release*, 2000, **64**, 193; P. van de Wetering, J. Y. Cherng, H. Talsma and W. E. Hennink, *J. Controlled Release*, 1997, **49**, 59.
37. E. S. Lee, K. Na and Y. H. Bae, *J. Controlled Release*, 2003, **91**, 103.
38. T. J. Martin, K. Prochazka, P. Munk and S. E. Webber, *Macromolecules*, 1996, **29**, 6071.
39. U. Borchert, U. Lipprandt, M. Bilang, A. Kimpfler, A. Rank, R. Peschka, R. Schubert, P. Lindner and S. Forster, *Langmuir*, 2006, **22**, 5843.
40. C. Giacomelli, L. Le Men, R. Borsali, J. Lai-Kee-Him, A. Brisson, S. P. Armes and A. L. Lewis, *Biomacromolecules*, 2006, **7**, 817.
41. M. C. Jones, M. Ranger and J. C. Leroux, *Bioconjugate Chem*., 2003, **14**, 774.
42. V. P. Sant, D. Smith and J.-C. Leroux, *J. Controlled Release*, 2004, **97**, 301.
43. J. Taillefer, M. C. Jones, N. Brasseur, J. E. van Lier and J. C. Leroux, *J. Pharm. Sci*., 2000, **89**, 52.
44. E. R. Gilles, T. B. Jonsson and J. M. Frechet, *J. Am. Chem. Soc*., 2004, **126**, 11936.
45. E. R. Gillies and J. M. Frechet, *Bioconjugate Chem*., 2005, **16**, 361.
46. Y. Bae, N. Nishiyama, S. Fukushima, H. Koyama, M. Yasuhiro and K. Kataoka, *Bioconjugate Chem*., 2005, **16**, 122.
47. A. Napoli, M. Valentini, N. Tirelli, M. Muller and J. A. Hubbell, *Nat. Mater*., 2004, **3**, 183.
48. P. Anton, J. Heinze and A. Laschewsky, *Langmuir*, 1993, **9**, 77.
49. Y. Takeoka, T. Aoki, K. Sanui, N. Ogata, M. Yokoyama, T. Okano, Y. Sakurai and M. Watanabe, *J. Controlled Release*, 1995, **33**, 79.
50. N. Rapoport, A. Marin and D. Christensen, *Drug Delivery Syst. Sci*., 2002, **2**, 37.
51. E. Ryschich, G. Huszty, H. Knaebel, M. Hartel, M. Buchler and J. Schmidt, *Eur. J. Cancer*, 2004, **40**, 1418; T. Szekeres, J. Sedlak and L. Novotny, *Curr. Med. Chem*., 2002, **9**, 759.
52. S. Vinogradov, E. Batrakova, S. Li and A. Kabanov, *Bioconjugate Chem*., 1999, **10**, 851.
53. M. Kursa, G. F. Walker, V. Roessler, M. Ogris, W. Roedl, R. Kircheis and E. Wagner, *Biocong. Chem*., 2003, **14**, 222.
54. M. Ogris, G. Walker, T. Blessing, R. Kircheis, M. Wolschek and E. Wagner, *J. Controlled Release*, 2003, **91**, 173.
55. C. Carlin, D. Simon, J. Mattison and B. Knowles, *Mol. Cell. Biol*., 1988, **8**, 25.
56. P. Järver and Ű. Langel, *Biochim. Biophys. Acta*, 2006, **1758**, 260.
57. V. P. Torchilin, *Adv. Drug Delivery Rev*., 2008, **60**, 548.
58. R. Savić, L. Luo, A. Eisenberg and D. Maysinger, *Science*, 2003, **300**, 615.
59. J. Nicolas, G. Mantovani and D. M. Haddleton, *Macromol. Rapid Commun*., 2007, **28**, 1083.
60. G. Thoma, J. T. Patton, J. L. Magnani, B. Ernst, R. Ohrlein and R. O. Duthaler, *J. Am. Chem. Soc*., 1999, **121**, 5919.
61. M. Okada, *Prog. Polym. Sci*., 2001, **26**, 67.
62. W. Ye, S. Wells and J. M. DeSimone, *J. Polym. Sci.: Part A: Polym. Chem*., 2001, **39**, 3841.
63. P. J. Julyan, L. W. Seymour, D. R. Ferry, S. Daryani, C. M. Boivin, J. Doran, M. David, D. Anderson, C. Christodoulou, A. M. Young, S. Hesslewood and D. J. Kerr, *J. Controlled Release*, 1999, **57**, 281.
64. K. Yasugi, T. Nakamura, Y. Nagasaki, M. Kato and K. Kataoka, *Macromolecues*, 1999, **32**, 8024.

65. Y. Nagasaki, K. Yasugi, Y. Yamamoto, A. Harada and K. Kataoka, *Biomacromolecules*, 2001, **2**, 1067.
66. D. W. Lim, Y. I. Yeom and T. G. Park, *Bioconjugate Chem.*, 2000, **11**, 1067.
67. E. Jule, Y. Nagasaki and K. Kataoka, *Langmuir*, 2002, **18**, 10334.
68. E. Jule, Y. Nagasaki and K. Kataoka, *Bioconjugate Chem.*, 2003, **14**, 177.
69. C. W. Cairo, J. E. Gestwicki, M. Kanai and L. L. Kiessling, *J. Am. Chem. Soc.*, 2002, **124**, 1615.
70. C. R. Bertozzi and L. L. Kiessling, *Science*, 2001, **291**, 2357.
71. V. Ladmiral, E. Melia and D. M. Haddleton, *European Polymer J.*, 2004, **40**, 431; S. G. Spain, M. I. Gibson and N. R. Cameron, *J. Polym. Sci. Part A: Polym. Chem.*, 2007, **45**, 2059.
72. M. Iijima, Y. Nagasaki, T. Okada, M. Kato and K. Kataoka, *Macromolecules*, 1999, **32**, 1140–1146.
73. K. Emoto, Y. Nagasaki and K. Kataoka, *Langmuir*, 1999, **15**, 5212.
74. Y. Y. Won, H. T. Davis and F. S. Bates, *Science*, 1999, **283**, 960.
75. Y. Li, B. S. Lokitz, S. P. Armes and C. L. McCormick, *Macromolecules*, 2006, **39**, 2726.
76. Q. G. Ma, E. E. Remsen, T. Kowalewski, J. Schaefer and K. L. Wooley, *Nano Letters*, 2001, **1**, 651.
77. K. L. Wooley, *J. Polym. Sci., Part A: Polym. Chem.*, 2000, **38**, 1397.
78. K. B. Thurmond, T. Kowalewski and K. L. Wooley, *J. Am. Chem. Soc.*, 1996, **118**, 7239.
79. H. Huang, E. E. Remsen, T. Kowalewski and K. L. Wooley, *J. Am. Chem. Soc.*, 1999, **121**, 3805.
80. R. K. O'Reilly, C. L. Hawker and K. L. Wooley, *Chem. Soc. Rev.*, 2006, **35**, 1068.
81. R. K. O'Reilly, M. J. Joralemon, C. J. Hawker and K. L. Wooley, *J Polym Sci. Part A: Polym. Chem.*, 2006, **44**, 5203.
82. E. S. Read and S. P. Armes, *Chem. Commun.*, 2007, 3021.
83. J. Liu, Y. Xiao and C. Allen, *J. Pharm. Sci.*, 2004, **93**, 132.
84. T. Nakanishi, S. Fukushima, K. Okamoto, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai and K. Kataoka, *J. Controlled Release*, 2001, **74**, 295.
85. T. G. Park, J. H. Jeong and S. W. Kim, *Adv. Drug Delivery Rev.*, 2006, **58**, 467.
86. N. Nishiyama and K. Kataoka, *Pharmacol. Therapeutics*, 2006, **112**, 630.
87. H. Cabral, N. Nishiyama and K. Kataoka, *J. Controlled Release*, 2007, **121**, 146.
88. C. Barner-Kowollik, T. P. Davis, J. P. A. Heuts, M. H. Stenzel, P. Vana and M. Whittaker, *J. Polym. Sci: Part A: Polym. Chem.*, 2003, **41**, 365; G. Moad, E. Rizzardo and S. H. Thang, *Aust. J. Chem.*, 2005, **58**, 379; S. Perrier and P. Takolpuckdee, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 5347; A. Favier and M.-T. Charreyre, *Macromol. Rapid Commun.*, 2006, **27**, 653.
89. L. Barner, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *Macromol. Rapid Commun.*, 2007, **28**, 539; C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Aust. J. Chem.*, 2006, **59**, 719.
90. T. Krasia, R. Soula, H. G. Börner and H. Schlaad, *Chem. Commun.*, 2003, 538.
91. M. S. Donavan, A. B. Lowe, T. A. Sanford and C. L. McCormick, *J. Polym. Sci. Part A: Polym. Chem.*, 2003, **41**, 1262.
92. M. J. Monteiro, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 5643.
93. M. Arotçaréna, B. Heise, S. Ishaya and A. Laschewsky, *J. Am. Chem. Soc.*, 2002, **124**, 3787.
94. Y.-C. Hu and C.-Y. Pan, *J. Polym. Sci. Part A: Polym. Chem.*, 2004, **42**, 4862.
95. J. Xu, J. He, D. Fan, W. Tang and Y. Yang, *Macromolecules*, 2006, **39**, 3753.
96. J. F. Quinn, L. Barner, C. Barner-Kowollik, E. Rizzardo and T. P. Davis, *Macromolecules*, 2002, **35**, 7620.
97. L. Albertin, M. H. Stenzel, C. Barner-Kowollik and T. P. Davis, *Polymer*, 2006, **47**, 1011.
98. D. B. Thomas, A. J. Convertine, R. D. Hester, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2004, **37**, 1735.
99. Y. A. Vasilieva, D. B. Thomas, C. W. Scales and C. L. McCormick, *Macromolecules*, 2004, **37**, 2728.
100. P. Vana, L. Albertin, T. P. Davis, L. Barner and C. Barner-Kowollik, *J. Polym. Sci. Part A: Polym. Chem.*, 2002, **40**, 4032.
101. X. Hao, M. H. Stenzel, C. Barner-Kowollik, T. P. Davis and E. Evans, *Polymer*, 2004, **45**, 7401.
102. K. H. Wong, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Aust. J. Chem.*, 2006, **59**, 539.
103. A. Laschewsky, M. Mertoglu, S. Kubowicz and A. F. Thűnemann, *Macromolecules*, 2006, **39**, 9337.
104. B. Y. K. Chong, T. P. L. Le, G. Moad, E. Rizzardo and S. H. Tang, *Macromolecules*, 1999, **32**, 2071.
105. C. Barner-Kowollik, J. F. Quinn, D. R. Morsley and T. P. Davis, *J. Polym. Sci., Part A: Polym. Chem.*, 2001, **39**, 1353–1365.
106. M. H. Stenzel, L. Cummins, G. E. Roberts, T. P. Davis, P. Vana and C. Barner-Kowollik, *Macromol. Chem. Phys.*, 2003, **204**, 1160.
107. M. L. Coote, E. I. Izgorodina, G. E. Cavigliasso, M. Roth, M. Busch and C. Barner-Kowollik, *Macromolecules*, 2006, **39**, 4584; A. Theis, M. H. Stenzel, T. P. Davis, M. L. Coote and C. Barner-Kowollik, *Aust. J. Chem.*, 2005, **58**, 437.
108. Selected publications on click chemistry, polymers: V. Ladmiral, G. Mantovani, G. J. Clarkson, S. Cauet, J. L. Irwin and D. M. Haddleton, *J. Am. Chem. Soc.*, 2006, **128**, 4823; G. Mantovani, V. Ladmiral, L. Tao and D. M. Haddleton, *Chem. Commun.*, 2005, 2089; J. A. Johnson, D. R. Lewis, D. D. Dıaz, M. G. Finn, J. T. Koberstein and N. J. Turro, *J. Am. Chem. Soc.*, 2006, **128**, 6564; R. Luxenhofer and R. Jordan, *Macromolecules*, 2006, **39**, 3509; T. Liebert, C. Hänsch and T. Heinze, *Macromol. Rapid Commun.*, 2006, **27**, 208; D. A. Ossipov and J. Hilborn, *Macromolecules*, 2006, **39**, 1709.
109. D. Quemener, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Chem. Commun.*, 2006, 5051.
110. L. Shi, T. M. Chapman and E. J. Beckman, *Macromolecules*, 2003, **36**, 2563.
111. Z. Ma and P. Lacroix-Desmazes, *J. Polym. Sci. Part A: Polym. Chem.*, 2004, **42**, 2405.
112. Z. Ma and P. Lacroix-Desmazes, *Polymer*, 2004, **45**, 6789.
113. C.-Y. Hong, Y.-Z. You and C.-Y. Pan, *J. Polym. Sci. Part A: Polym. Chem.*, 2004, **42**, 4873.
114. Y. Li, B. Lokitz and C. L. McCormick, *Macromolecules*, 2006, **39**, 81.
115. X. Xu, Z. Jia, R. Sun and J. Huang, *J. Polym. Sci. Part A: Polym. Chem.*, 2006, **44**, 4396.
116. G. Pound, F. Aguesse, J. B. McLeary, R. F. M. Lange and B. Klumperman, *Macromolecules*, 2007, **40**, 8861.
117. J. Bang, S. H. Kim, E. Drockenmuller, M. J. Misner, T. P. Russell and C. J. Hawker, *J. Am. Chem. Soc.*, 2006, **128**, 7622.
118. H. De Brower, M. A. J. Schellekens, B. Klumperman, M. J. Monteiro and A. L. German, *J. Polym. Sci. Part A: Polym. Chem.*, 2000, **38**, 3596.
119. N. Kawahara, S.-I. Kojoh, S. Matsuo, H. Kaneko, T. Matsugi, J. Saito and N. Kashiwa, *Polymer Bull.*, 2006, **57**, 805.
120. R. Godoy-Lopez, C. Boisson, F. D'Agosto, R. Spoutz, F. Boisson, D. Gigmes and D. Bertin, *Macromol. Rapid Commun.*, 2006, **27**, 173.
121. T. S. C. Pai, C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Polymer*, 2004, **45**, 4383.
122. M. Hales, C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Langmuir*, 2004, **20**, 10809.
123. Y. You, C. Hong, W. Wang, W. Lu and C. Pan, *Macromolecules*, 2004, **37**, 9761.
124. M. K. Mahanthappa, F. S. Bates and M. A. Hillmeyer, *Macromolecules*, 2005, **38**, 7890.
125. K. J. Thurecht, A. M. Gregory, S. Villarroya, J. Zhou, A. Heise and S. M. Howdle, *Chem. Commun.*, 2006, 4383.
126. S. Sinnwell, A. J. Inglis, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *Chem. Commun.*, 2008.
127. R. Hoogenboom, U. S. Schubert, W. van Camp and F. Du Prez, *Macromolecules*, 2005, **38**, 7653.
128. D. Beattie, K. H. Wong, C. Williams, L. A. Poole-Warren, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Biomacromolecules*, 2006, **7**, 1072.
129. B.-Q. Zhang, G.-D. Chen, C.-Y. Pan, B. Luan and C.-Y. Hong, *J. Appl. Polym. Sci.*, 2006, **102**, 1950.

130. M. Save, M. Manguian, C. Chassenieux and B. Charleux, *Macromolecules*, 2005, **38**, 280.
131. S. Garnier and A. Laschewsky, *Macromolecules*, 2005, **38**, 7580.
132. M. H. Stenzel, C. Barner-Kowollik, T. P. Davis and H. M. Dalton, *Macromol. Bioscience*, 2004, **4**, 445.
133. K. H. Wong, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Polymer*, 2007, **48**, 4950.
134. M. H. Stenzel, Complex Architecture Design *via* the RAFT Process: Scope, Strengths and Limitations, in *"Handbook of RAFT polymerization"*, ed. C Barner-Kowollik, Wiley, 2008, ISBN: 978-3-527-31924-4.
135. M. Mertoglu, S. Garnier, A. Laschewsky, K. Skrabania and J. Storsberg, *Polymer*, 2005, **46**, 7726.
136. L. Zhang, L. T. U. Nguyen, J. Bernard, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Biomacromolecules*, 2007, **8**, 2890.
137. S. Garnier and A. Laschewsky, *Macromolecules*, 2005, **38**, 7580.
138. A. Laschewsky, M. Mertoglu, S. Kubowicz and A. F. Thűnemann, *Macromolecules*, 2006, **39**, 9337.
139. Z. Cheng, X. Zhu, E. T. Kang and K. G. Neoh, *Langmuir*, 2005, **21**, 7180.
140. L. T. U. Nguyen, K. Eagles, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *J. Polym. Sci. Part A: Polym. Chem.*, 2006, **44**, 4372.
141. G. Moad, J. Chiefari, J. Krstina, A. Postma, R. T. A. Mayadunne, E. Rizzardo and S. H. Thang, *Polym. Int.*, 2000, **49**, 993.
142. D. C. Wan, K. Satoh, M. Kamigaito and Y. Okamoto, *Macromolecules*, 2005, **38**, 10397.
143. R. Devasia, R. L. Bindu, R. Borsali, N. Mougin and Y. Gnanou, *Macromol. Symp.*, 2005, **229**, 8.
144. A. Postma, T. P. Davis, R. A. Evans, G. Li, G. Moad and M. O'Shea, *Macromolecules*, 2006, **39**, 5293.
145. M. H. Stenzel, L. Cummins, G. E. Roberts, T. P. Davis, P. Vana and C. Barner-Kowollik, *Macromol. Chem. Phys.*, 2003, **204**, 1160.
146. M. H. Stenzel, T. P. Davis and C. Barner-Kowollik, *Chem. Commun.*, 2004, **13**, 1546.
147. J. Bernard, A. Favier, L. Zhang, A. Nilasaroya, C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Macromolecules*, 2005, **38**, 5475.
148. D. Boschmann and P. Vana, *Polym. Bull.*, 2005, **53**, 231.
149. D. Charmont, P. Corpart, H. Adam, S. Z. Zard, T. Biadetti and G. Bouhadir, *Macromol. Symp.*, 2000, **150**, 23.
150. E. Rizzardo, J. Chiefari, R. T. A. Mayadunne, G. Moad and S. H. Thang, *ACD Symp. Ser.*, 2000, **768**, 278.
151. A. Favier, C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Macromol. Chem. Phys.*, 2004, **205**, 925.
152. B. S. Lokitz, A. J. Convertine, R. G. Ezell, A. Heidenreich, Y. Li and C. McCormick, *Macromolecules*, 2006, **39**, 8594.
153. Y. Zhou, K. Jiang, Q. Song and S. Liu, *Langmuir*, 2007, **23**, 13076.
154. C. Yang and Y.-L. Cheng, *J. Appl. Polym. Sci.*, 2006, **102**, 1191.
155. T. Tang, V. Castelletto, P. Parras, I. W. Hamley, S. M. King, D. Roy, S. Perrier, R. Hoogenboom and U. S. Schubert, *Macromol. Chem. Phys.*, 2006, **207**, 1718.
156. M. Nakayama and T. Okano, *Biomacromolecules*, 2005, **6**, 2320.
157. M. Nuopponen, J. Ojala and H. Tenhu, *Polymer*, 2004, **45**, 3643.
158. A. B. Lowe, M. Torres and R. Wang, *J. Polym. Sci., Part A: Polym. Chem.*, 2007, **45**, 5864.
159. J. Bernard, X. Hao, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Biomacromolecules*, 2006, **7**, 232.
160. C. M. Schilli, M. Zhang, E. Rizzardo, S. H. Tang, B. Y. K. Chong, K. Edwards, G. Karlsson and A. H. E. Műller, *Macromolecules*, 2004, **37**, 7861.
161. Z. Ge, Y. Cai, J. Yin, J. Rao and S. Liu, *Langmuir*, 2007, **23**, 1114.
162. Y.-Z. You, Q.-H. Zhou, D. S. Manickam, L. Wan, G.-Z. Mao and D. Oupicky, *Macromolecules*, 2007, **40**, 8617.
163. Y. Li, B. S. Lokitz and C. L. McCormick, *Angew. Chem. Int. Ed.*, 2006, **45**, 5792.
164. S.-I. Yusa, Y. Shimada, Y. Mitsukami, T. Yamamoto and Y. Morishima, *Macromolecules*, 2004, **37**, 7507.
165. Y. Zheng, S. Luo and S. Liu, *Macromolecules*, 2005, **38**, 9813.
166. A. J. Convertine, B. S. Lokitz, Y. Vasileva, L. J. Myrick, C. W. Scales, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2006, **39**, 1724.
167. B. Liu and S. Perrier, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 3643.
168. L. Zhang, J. Bernard, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Macromol. Rapid Commun.*, 2008, **29**, 123.
169. M. Arotçaréna, B. Heise, S. Ishaya and A. Laschewsky, *J. Am. Chem. Soc.*, 2002, **124**, 3787.
170. M. Nakayama and T. Okano, *Biomacromolecules*, 2005, **6**, 2320.
171. R. Nojima, T. Sato, X. Qiu and F. M. Winnik, *Macromolecules*, 2008, **41**, 292.
172. S.-I. Yusa, K. Fukuda, T. Yamamoto, K. Ishihara and Y. Morishima, *Biomacromolecules*, 2005, **6**, 663.
173. M. H. Stenzel, C. Barner-Kowollik, T. P. Davis and H. M. Dalton, *Macromol. Biosci.*, 2004, **4**, 445.
174. Y.-Z. You, D. S. Manickam, Q.-H. Zhou and D. Oupicky, *J. Controlled Release*, 2007, **122**, 217.
175. A. B. Lowe and C. L. McCormick, *Prog. Polym. Sci.*, 2007, **32**, 283.
176. A. B. Lowe, B. S. Sumerlin and C. L. McCormick, *Polymer*, 2003, **44**, 6761.
177. L. Albertin, M. H. Stenzel, C. Barner-Kowollik, L. J. R. Foster and T. P. Davis, *Macromolecules*, 2005, **38**, 9075.
178. L. Albertin, M. H. Stenzel, C. Barner-Kowollik, L. J. R. Foster and T. P. Davis, *Macromolecules*, 2004, **37**, 7530.
179. L. Albertin, C. Kohlert, M. Stenzel, J. L. R. Foster and T. P. Davis, *Biomacromolecules*, 2004, **5**, 255.
180. S. G. Spain, L. Albertin and N. R. Cameron, *Chem. Commun.*, 2006, 4198.
181. T.-Y. Guo, P. Liu, J.-W. Zhu, M.-D. Song and B.-H. Zhang, *Biomacromolecules*, 2006, **7**, 1196.
182. L. Albertin and N. R. Cameron, *Macromolecules*, 2007, **40**, 6082.
183. M. Al-Bagoury, K. Buchholz and E.-J. Yaacoub, *Polym. Adv. Technol.*, 2007, **18**, 313.
184. J. Bernard, A. Favier, L. Zhang, A. Nilasaroya, C. Barner-Kowollik, T. P. Davis and M. H. Stenzel, *Macromolecules*, 2005, **38**, 5475.
185. A. B. Lowe and R. Wan, *Polymer*, 2007, **48**, 2221.
186. V. Ramiah, H. Matahwa, W. Weber, J. B. McLeary and R. D. Sanderson, *Macromol. Symp.*, 2007, **255**, 70.
187. S. R. Simon Ting, A. Granville, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *Aust. J. Chem.*, 2007, **60**, 405.
188. R. Narain, A. Housni, G. Gody, P. Boullanger, M.-T. Charreyre and T. Delair, *Langmuir*, 2007, **23**, 12835.
189. Z. Özyrürek, H. Komber, S. Gramm, D. Schmaljohann, A. H. E. Müller and B. Voit, *Macromol. Chem. Phys.*, 2007, **208**, 1035.
190. A. M. Granville, D. Quémener, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Macromol. Symp.*, 2007, **255**, 81.
191. C. F. Brewer and R. D. Brown, *Biochemistry*, 1979, **18**, 2555.
192. M. Bathfield, F. D'Agosto, R. Spitz, M.-T. Charreyre and T. Delair, *J. Am. Chem. Soc.*, 2006, **128**, 2546.
193. N.-Y. Xiao, A.-L. Li, H. Liang and J. Lu, *Macromolecules*, 2008, **41**, 2374.
194. G. Gody, P. Boullanger, C. Ladavière, M. T. Charreyre and T. Delair, *Macromol. Rapid. Commun*, 2008, early view.
195. A. Aqil, H. Qiu, J.-F. Greisch, R. Jerome, E. De Pauw and C. Christine, *Polymer*, 2008, **49**, 1145.
196. C.-Y. Hong and C.-Y. Pan, *Macromolecules*, 2006, **39**, 3517.
197. J. Liu, V. Bulmus, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Macromol. Rapid. Commun.*, 2007, **28**, 305.
198. A. N. Zelikin, G. K. Such, A. Postma and F. Caruso, *Biomacromolecules*, 2007, **8**, 2950.
199. J. Liu, V. Bulmus, D. L. Herlambang, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Angew. Chem.*, 2007, **46**, 3099.
200. C. Boyer, J. Liu, V. Bulmus, T. P. Davis, M. H. Stenzel and C. Barner-Kowollik, *J. Am. Chem. Soc.*, 2007, **129**, 7145.
201. J. Hentschel, M. G. J. ten Cate and H. G. Boerner, *Macromolecules*, 2007, **40**, 9224.
202. M. G. J. ten Cate and H. G. Boerner, *Macromol. Chem. Phys.*, 2007, **208**, 1437.
203. Y. Zhao and S. Perrier, *Chem. Commun.*, 2007, **41**, 4294.
204. P. Relógio, M.-T. Charreyre, J. P. S. Farinha, J. M. G. Martinho and C. Pichot, *Polymer*, 2004, **45**, 8639.
205. A. Favier, F. D'Agosto, M. T. Charreyre and C. Pichot, *Polymer*, 2004, **45**, 8639.
206. B. de Lambert, C. Chaix, M. T. Charreyre, T. Martin, A. Aigoui, A. Perrin-Rubens and C. Pichot, *Bioconjugate Chem.*, 2005, **15**, 265.

207. E. N. Savariar and S. Thayumanavan, *J. Polym. Sci.: Part A: Polym. Chem.*, 2004, **42**, 6340.
208. J. Mallinamadugu, K. Yanjarappa, K. V. Gujraty, A. Joshi, A. Saraph and R. S. Kane, *Biomacromolecules*, 2006, **7**, 1665.
209. J. Hwang, R. C. Li and H. D. Maynard, *J. Controlled Release*, 2007, **122**, 279.
210. R. C. Li, J. Hwang and H. D. Maynard, *Chem. Commun.*, 2007, 3631.
211. H. Mori, K. Sutoh and T. Endo, *Macromolecules*, 2005, **38**, 9055.
212. H. Mori, H. Iwaya, A. Nagai and T. Endo, *Chem. Commun.*, 2005, 4872.
213. H. Mori, H. Iwaya and T. Endo, *Macromol. Chem. Phys.*, 2007, **208**, 1908.
214. X. Zhang, J. Li, W. Li and A. Zhang, *Biomacromolecules*, 2007, **8**, 3557.
215. P. De, S. R. Gondi and B. S. Sumerlin, *Biomacromolecules*, 2008, **9**, 1064.
216. L. He, E. S. Read, S. P. Armes and D. J. Adams, *Macromolecules*, 2007, **40**, 4429.
217. Y. Maki, H. Mori and T. Endo, *Macromol. Chem. Phys.*, 2007, **208**, 2589.
218. B. S. Lokitz, A. J. Convertine, R. G. Ezell, A. Heidenreich, Y. Li and C. L. McCormick, *Macromolecules*, 2006, **39**, 8594.
219. B. S. Lokitz, A. W. York, J. E. Stempka, N. D. Treat, Y. Li, W. L. Jarrett and C. L. McCormick, *Macromolecules*, 2007, **40**, 6473.
220. Y. Li, B. S. Lokitz and C. L. McCormick, *Angew. Chem., Int. Ed.*, 2006, **45**, 5792.
221. S. Harrisson and K. L. Wooley, *Chem. Commun.*, 2005, 3259.
222. Y. Li, B. S. Lokitz and C. L. McCormick, *Macromolecules*, 2006, **39**, 81.
223. Y. Li, B. S. Lokitz, S. P. Armes and C. L. McCormick, *Macromolecules*, 2006, **39**, 2726.
224. J. Zhang, X. Jiang, Y. Zhang, Y. Li and S. Liu, *Macromolecules*, 2007, **40**, 9125.
225. Q. Zheng, G.-H. Zheng and C.-Y. Pan, *Polym. Int.*, 2006, **55**, 1114.
226. L. P. Yang and C.-Y. Pan, *Aust. J. Chem.*, 2006, **59**, 733.
227. G. Zheng and C.-Y. Pan, *Macromolecules*, 2006, **39**, 95.
228. L. Zhang, K. Katapodi, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *J. Polym. Sci. Part A: Polym. Chem.*, 2006, **44**, 2177.
229. P. Zhang, Q. Liu, A. Qing, J. Shi and M. Lu, *J. Polym. Sci.: Part A: Polym. Chem.*, 2006, **44**, 3312.
230. L. Zhang, J. Bernard, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Macromol. Rapid Commun.*, 2008, **29**, 123.
231. L. Zhang and M. H. Stenzel, *Biomacromolecules*, 2008, submitted.
232. L. Barner and S. Perrier, "Polymers with well-defined endgroups *via* RAF", , in *"Handbook of RAFT polymerization"*, ed. C Barner-Kowollik, Wiley-VCH, 2008.
233. T. L. U. Nguyen, B. Farrugia, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *J. Pol. Sci. Part A: Polym. Chem.*, 2007, **45**, 3256.
234. Y. Liu, J. He, J. Xu, D. Fan, W. Tang and Y. Yang, *Macromolecules*, 2005, **38**, 10332.
235. J. Xu, J. He, D. Fan, W. Tang and Y. Yang, *Macromolecules*, 2006, **39**, 3753.
236. T. Arita, M. Buback and P. Vana, *Macromolecules*, 2005, **38**, 7935.
237. T. L. Legge, A. Slark and S. Perrier, *J. Polym. Sci., Part A: Polym. Chem.*, 2006, **59**, 755.
238. B. Chong, G. Moad, E. Rizzardo, M. Skidmore and S. H. Thang, *Aust. J. Chem.*, 2006, **59**, 755.
239. F. Cerreta, A. M Lenocher, C. Leriverrend, P. Metzner and T. N. Pham, *Bull. Soc. Chim. Fr.*, 1995, **132**, 67.
240. L. Lu, H. J. Zhang, N. F. Yang and Y. L. Cai, *Macromolecules*, 2006, **39**, 3770.
241. J. F. Quinn, T. P. Davis, L. Barner and C. Barner-Kowollik, *Polymer*, 2007, **48**, 6467.
242. S. Perrier, P. Takolpuckdee and C. A. Mars, *Macromolecules*, 2005, **38**, 2033.
243. G. Moad, Y. K. Chong, A. Postma, E. Rizzardo and S. H. Thang, *Polymer*, 2005, **46**, 8456.
244. Y. K. Chong, G. Moad, E. Rizzardo and S. Thang, *Macromolecules*, 2007, **40**, 4446.
245. D. B. Thomas, A. J. Covertine, R. D. Hester, A. B. Lowe and C. L. McCormick, *Macromolecules*, 2004, **37**, 1735.
246. R. T. A. Mayadunne, J. Jeffery, G. Moad and E. Rizzardo, *Macromolecules*, 2003, **36**, 1505; R. T. A. Mayadunne, E. Rizzardo, J. Chiefari, J. Krstine, G. Moad, A. Postma and S. H. Thang, *Macromolecules*, 2000, **33**, 243.
247. M. L. Llauro, J. F. Boisson, F. Delolme, C. Ladaviere and J. Claverie, *J. Polym. Sci., Part A: Polym. Chem.*, 2004, **42**, 5439.
248. D. L. Patton, M. Mullings, T. Fulghum and R. C. Advincula, *Macromolecules*, 2005, **38**, 8597.
249. J. Li, W.-D. He and X.-L. Sun, *J. Polym. Sci. Part A: Polym. Chem.*, 2007, **45**, 5156.
250. Z. M. Wang, J. P. He, Y. F. Tao, L. Yang, H. J. Jiang and Y. L. Yang, *Macromolecules*, 2003, **36**, 7446.
251. C. W. Scales, A. J. Convertine and C. L. McCormick, *Biomacromolecules*, 2006, **7**, 1389.
252. X. P. Qiu and F. M. Winnik, *Macromol. Rapid Commun.*, 2006, **27**, 1648.
253. F. Segui, X.-P. Qiu and F. M. Winnik, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 314.
254. D. Sutton, N. Nasongkla, E. Blanco and J. Gao, *Pharm. Res.*, 2007, **24**, 1029.
255. R. Duncan, *Nat. Rev., Drug Discovery*, 2003, **2**, 347.
256. T. P. Le, G. Moad, E. Rizzardo and S. Thang, *PCT Int Appl. WO 980 1478*, 1998.