

## INVITED REVIEW

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## Recent developments in atom transfer radical polymerization initiators for development of polymer-protein bioconjugates

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### ABSTRACT

One of the major challenges in modern synthetic polymer chemistry is to synthesize end defined polymers of different end functionality with predetermined uniform molecular weight. End functionalized polymers/copolymers basically in block and grafting form are having several potential applications in biomedical areas in the form of surface modifications, coatings, adhesives, and in order to increase the biocompatibility of polymeric blends. Among the existing controlled radical polymerization (CRP) methods for synthesis of these functional polymers, the atom transfer radical polymerization (ATRP) is one of the powerful techniques. The functional groups in these polymers can be easily introduced at the chain ends through functionalized ATRP initiators. A number of ATRP initiators have been developed in polymer science to develop defined polymer-protein bioconjugates. This critical review basically focuses on different types of ATRP initiators and their mechanisms used in the synthesis of polymer-protein bioconjugates.

**Key words:** Atom transfer radical polymerization, polymeric initiators, block polymer, graft polymer, star polymer, polymer-protein bioconjugates

## INTRODUCTION

The precise synthesis of polymer employs the concept of living polymerization, in which the chain termination reactions are minimized and as a result simultaneous chain length can be obtained via nearly instantaneous initiation. A combination of immediate initiation and an absence of termination are the ideal conditions for a living polymerization. Practically, conventional reactions proceed via slow initiation, resulting in short polymeric chains and become dead at a given point of time. To obtain the ideal conditions, various controlled/living radical systems have

been developed and they are usually progressed via formation of an intermediate active propagating species, which have a dynamic equilibrium between growing and dormant species with similar anionic, cationic, coordination and ring-opening polymerization systems. The establishment of such a dynamic balance between propagating radicals and various dormant species is a critical step to all controlled radical polymerization (CRP) systems (Fisher 2004; Goto & Fukuda, 2004). In these systems, radicals may either be reversibly involved in deactivation/activation reactions or can be reversibly transferred to growing radicals.

### Abbreviations:

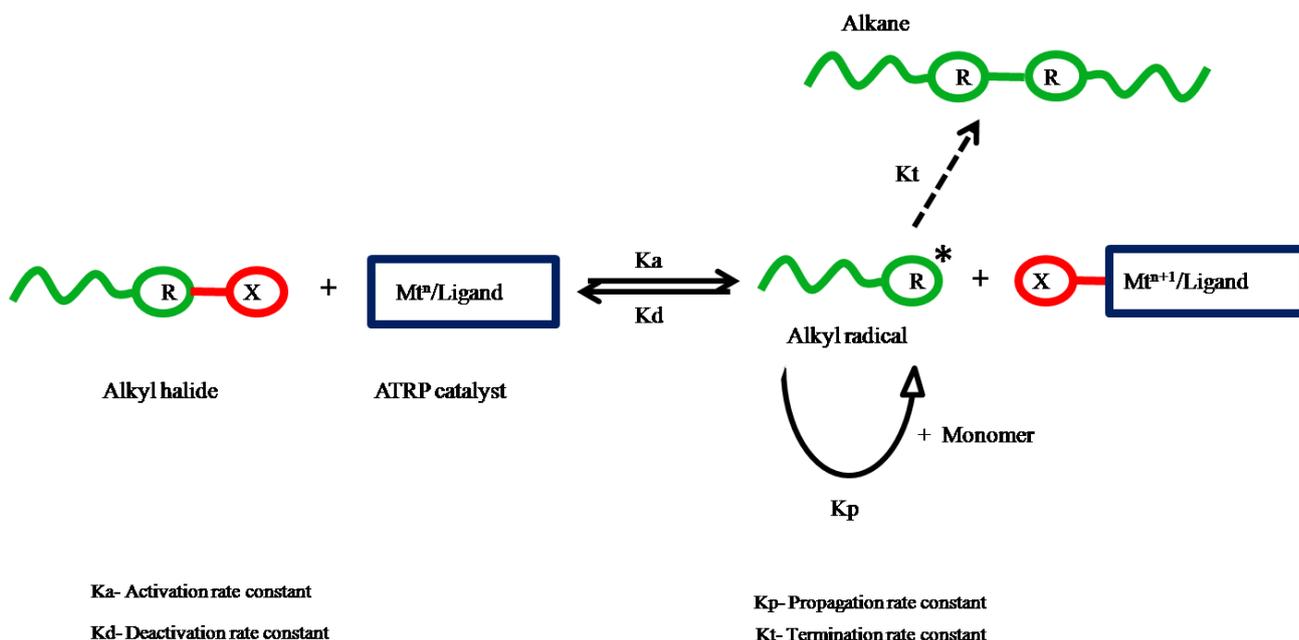
AGET, Activators generated electron transfer; ATRP, Atom transfer radical polymerization; BAB, Poly[(t-BuA)-b-(Sty)-b-(t-BuA)]; CRP, Control radical polymerization; CYCLAM, Tetraazacyclotetradecane; DEA, *N*, *N*-diethylaminoethylmethacrylate; DMA, *N*, *N*-dimethylacrylamide; diPENTA, Dipentaerythritol; diTMP, Ditrithymolpropane; DVB, Divinyl benzene; E2BB, Ethyl-2-bromoisobutyrate; HEC, O-(2-hydroxyethyl) cellulose; HEC-g-PDMA, Hydroxyethylcellulose-graft-Poly(*N,N* dimethylacrylamide); HEATMS, Trimethylsilyloxyethyl acrylate; ICAR, Initiators for continuous activator regeneration; Me6[14]aneN4, Macrocyclotetraamine; MPCs, 2, 5-bis[(4-methoxyphenyl)oxycarbonyl]styrene; MPPM, Polypropylene membrane; NMP, Nitroxide mediated polymerization; PBiBEA, Poly(2-bromoisobutyryloxyethyl acrylate); PEOX, Poly(3-ethyl-3-hydroxymethyloxetane); PFS, Poly(pentafluorostyrene); P(GMA), Poly(glycidyl methacrylate); PMMA, Poly(methyl methacrylate); P(4-MS), Poly(4-methyl styrene); PNiPAAm, Poly-*N*-isopropylacrylamide; PNIPAm-*b*-PZLys, Poly(*N*-isopropylacrylamide)-*b*-poly(*Z*-L-lysine); PrBA-*b*-PS, Poly(tert-butyl acrylate)-*b*-polystyrene; P4VP, Polyvinylpyrrolidone; PS, Polystyrene; PU, Polyurethane; RAFT, Reversible addition fragmentation transfer; ROP, Ring opening polymerization; TREN, Tris[2-aminoethyl]amine; TFMS, 2, 3, 5, 6-tetrafluoro-4-methoxystyrene; t-BuA, tert-butyl acrylate; VBC, 4-vinylbenzyl chloride.

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The living approach is based on the persistent radical effect (PRE) (Tang et al., 2006a; Tang et al., 2006b). The PRE is a special kinetic parameter, which provides self-regulation in few CRP systems. In PRE, the propagating radicals are rapidly involved in the deactivation reaction by chemical species and dormant species are activated either spontaneously, in presence of temperature, or an appropriate catalyst (as transition metals in atom transfer radical polymerization (ATRP)). On the other hand, sometimes the active radicals can also be terminated. Moreover, constant radicals are not terminated with each other because they are only reversibly cross reacting with growing species. Thus, propagating radical's termination takes place in conditions of irreversible accumulation of chemical species. Furthermore, the concentration of chemical species progressively increases with time during the reaction. Therefore, the concentrations of radicals as well as the probability of termination decreases with time and growing radicals are dominantly reacting with chemical species species, which are normally present in higher concentration (Braunecker & Matyjaszewski, 2007).

In existing LRP systems, the ATRP has emerged as one of the most powerful techniques for well-defined and controlled polymerization (Pokorski et al., 2011; Hu et al., 2012; Siegwarta et al., 2012; Jung & Theato, 2013). It was

first reported in 1995 and independently discovered by Mitsuo Sawamoto (Kato et al., 1995), Krzysztof Matyjaszewski, and Jin-Shan Wang (Wang et al., 1995). From mechanism point of view, ATRP comes from atom transfer radical addition reaction (ATRA) (Pintauer & Matyjaszewski, 2008). ATRA is a well-known organic reaction, in which halide species undergo homolytic cleavage, resulting in halogen atom that is transferred to a transition metal complex and forms a free radical, which reacts with unsaturated molecules (generally containing vinyl group). It is fast and irreversibly deactivated by back-transfer of halogen atom from the metal complex to organic radical species. In comparison to ATRA, ATRP has a system in which the deactivated species formed can be again reactivated and allowed for further growth of the polymeric chain and as a result polymers with controlled chain length can be synthesized. The selection of a suitable catalyst is an important factor in ATRP reaction, which enables the system to establish a reversible equilibrium between propagating and dormant species, as well as in enhancing the rate of initiation by minimizing the possibility of expected radical-radical termination for controlled growth of polymers (Matyjaszewski, 1995) (Figure 1).



**Figure 1.** Schematic presentation of Atom transfer radical polymerization (ATRP). The alkyl halide is activated (rate constant  $K_a$ ) in alkyl radical  $R^*$  in presence of ATRP catalyst and sometimes it can be deactivated (rate constant  $K_d$ ) or terminated in alkane (rate constant  $K_t$ ).

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The catalyst of ATRP is generally a transition metal complex, in which the ligand determines the reactivity of metal complex. In ATRP catalyst, a variety of transition metals have been used such as Ti (Kabachii et al., 2003), Mo (Brandts et al., 1999), Re (Kotani et al., 1999), Fe (Matyjaszewski et al., 1997), Ru (Kato et al., 1995), Os (Braunecker et al., 2005), Co (Wang et al., 2003), Ni (Uegaki et al., 1997), Cu (Wang et al., 1995) etc. Out of them, Cu metal complexes have been proved as an efficient catalyst for a broad range of monomers in diverse reaction media. Moreover, ATRP have some advantages over other CRP techniques due to the commercial availability of all required ATRP reagents.

In ATRP, ligand of transition metal complexes strongly affects the solubility of the catalyst complex, and accordingly the concentration of activators and deactivators in the reaction medium also get affected (Gao & Matyjaszewski, 2009). Therefore, the dynamic equilibrium can be easily modulated by modifying the associated ligand of the catalyst (Matyjaszewski & Xia, 2001). Generally, nitrogen-based ligands are employed in Cu ATRP based catalysts which include tris[2-aminoethyl]amine (TREN) (Xia et al., 1998), pyridine imine (Haddleton et al., 1997), and tetraazacyclotetradecane (CYCLAM) (Konak et al., 2002). In comparison to other CRP techniques such as nitroxide mediated polymerization (NMP), reverse addition fragment chain transfer (RAFT) polymerization, ATRP has a simple and efficient pathway to synthesis of polymers with well-defined chain (Coessens et al., 2001). Moreover, nowadays ATRP has also been used to synthesize polymers efficiently with complex architectures, such as block, graft, star polymers and end functionalized polymers for peptide/protein bioconjugation (Davis & Matyjaszewski, 2002). Furthermore, low molecular weight polymers have been also synthesized by ATRP (Min & Matyjaszewski, 2009).

**POLYMER-PROTEIN BIOCONJUGATES**

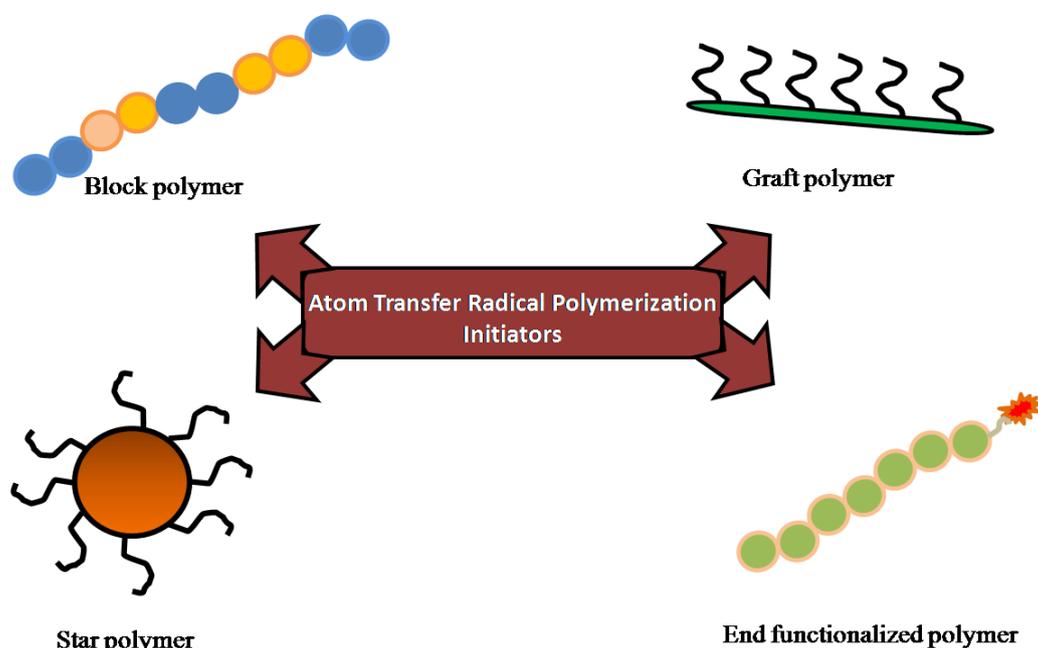
In life sciences, conjugation of polymer and protein is a great tool for development of novel therapeutics in pharmaceuticals, efficient separation of products in downstream processing, cells/biomolecules separation, development of clinical diagnostics methods and immunoassays. First time, Abuchowski and colleagues have developed polyethylene glycol (PEG) based protein bioconjugates for enhancing stability of protein molecules (Abuchowski et al., 1977). Various PEG based therapeutics

like L-asparaginase-PEG, growth hormone-PEG, interferon-PEG are commercialized available. Though, polymers helps in protects functional and structural stability of protein in these bioconjugates but few limitations have been reported with non-specific bioconjugation. In order to improve and achieve homogenous polymer protein bioconjugates, atom transfer radical polymerization has provided a new synthetic tool (Audouin et al., 2012; Broyer et al., 2012). In ATRP, choice of initiators is an important parameter. Therefore, a number of ATRP initiators have been developed for the synthesis of different polymeric architectures like block, star, graft and end functionalize polymer (Figure 2). They are described in detail in the following sections.

**Block polymers**

Nowadays, ATRP is one of the most rapidly growing techniques in polymer chemistry with numerous possibilities for polymerization of different functional monomers in a controlled manner, extensive use of initiators with functional groups, and end group functionalization of polymer. Additionally, ATRP has also appeared as an important tool in chemistry with enormous design flexibility providing a complete control over polymer composition, topology, and functionality (Min & Matyjaszewski, 2009). Like other LRP techniques, in ATRP the R-chain (alkyl moiety) terminus of initiator is an important factor in designing an activator, which is most often an alkyl halide with an activated substituent group in the R-group, which initiates the reaction through homolytic cleavage in the presence of a catalyst. An ester group is a better activator because it easily undergoes homolytic cleavage. Therefore R-haloester based chemical compounds are commonly used as ATRP initiators. The functionality incorporated in polymer via an ester linkage is usually susceptible to hydrolysis, therefore this limitation can be overcome by using an amide linkage in R-halo amide based initiators instead of ester (Baek et al., 2002; Limer & Haddleton, 2006). The uses of amide based initiators are nowadays considered as ATRP initiators for synthesis of polymer in a well-controlled manner with narrow molecular weight distribution. For the first time, Haddleton's laboratory used amide based ATRP initiators in synthesis of block polymers of various methacrylates and styrene monomers (Limer & Haddleton, 2006), followed by the work of Baek and colleagues (Baek et al., 2002), who synthesized poly(methyl methacrylate) with a narrow polydispersity index (PDI) using *N,N*-dimethyl-2 bromopropanamide as an initiator.

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**Figure 2.** Schematic presentation of important application of atom transfer radical polymerization (ATRP) based initiators in development of block, graft, star and end functionalized polymer.

Moreover, Matyjaszewski's team has studied the polymerization of methacrylamides using model R-haloamide based initiators, to obtain well-defined block copolymers (Teodorescu & Matyjaszewski, 1999). Furthermore, chloropropionamides as an initiator was used in polymerization of *N*-isopropylacrylamide monomers with small polydispersity index, to study the influence of end group functionalization on thermal property of polymer (Xia et al., 2006). In this direction, Venkataraman and their colleagues have synthesized block polymer by using amino acid based initiator, due to biological relevancy, commercial availability, amphiphilic nature, and simplicity. They have used valine as the precursor for synthesis of functional initiators and synthesized poly(*tert*-butyl acrylate)-*b*-polystyrene (*Pt*BA-*b*-PS) (4) diblock copolymer (Venkataraman & Wooley, 2006). Recently, a hydrolysis-resistant amide-linkage heterofunctional based initiator was used in the synthesis of well-defined rod-coil block copolymers poly(*N*-isopropylacrylamide)-*b*-poly(*Z*-L-lysine) (PNIPAm-*b*-PZLYs) by combination of ATRP and amine hydrochloride mediated ring-opening polymerization (ROP) techniques (Huang & Chang, 2008).

Block copolymers based on poly(pentafluorostyrene) (PFS) in various numbers and different lengths of polystyrene have been synthesized through ATRP. Di- and triblock copolymers with varying amounts of PFS have also been synthesized by employing either 1-phenylethylbromide or 1, 4-dibromoxylene as ATRP initiators. Diverse bromo(ester) (macro)initiators were also devised and involved in the preparation of fluorinated pentablock as well as amphiphilic triblock copolymers having a central polyether. Various polymer designs such as amphiphilic starshaped fluoropolymers, hydrophobic fluorinated nanoparticles, and segmented fluorinated star-shaped block copolymers have been further designed by use of different multifunctional initiators. A number of monosubstituted styrenes, such as 4-fluoro- and 4-trimethylfluorostyrene, have also been polymerized by ATRP (Qiu & Matyjaszewski, 1997). More recently, the fully phenyl fluorinated styrene, 2, 3, 4, 5, 6-pentafluorostyrene (FS), has also been polymerized by ATRP (Jankova & Hvilsted, 2003). Efficient and fast polymerization of FS has been successfully followed by ATRP of tetrafluoromethoxystyrene (TFMS) (Hvilsted et al., 2003).

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Furthermore, a newly synthesized highly fluorinated fluoroalkoxy styrene monomers, 2, 3, 5, 6-tetrafluoro-4-(2, 2, 3, 3, 3-pentafluoropropoxy)-styrene and 2, 3, 5, 6-tetrafluoro-4-(2, 2, 3, 3, 4, 4, 5, 5, 6, 6, 7, 7, 8, 8, 8-pentadecafluorooctoxy) -styrene were found to be of good application in synthesis of low surface energy materials (Borkar et al., 2004).

In block polymer synthesis, researchers have also tried functional polymeric macroinitiators for example, bifunctional polystyrene macroinitiators, having various molecular weights synthesized by ATRP initiated with bifunctional initiator 1, 3-bis(1-methyl-1(2, 2, 2-trichloroethoxy) carbonylamino ethylbenzene in conjunction with CuCl as a catalyst and polyamine ligands. These macroinitiators were later used for ATRP of tert-butyl acrylate (t-BuA), giving BAB triblocks poly[(t-BuA)-*b*-(Sty)-*b*-(t-BuA)] as precursor of amphiphilic polymers. Both the polymerization steps proceeded in a controlled manner with linear semi-logarithmic conversion plots and lengths of blocks following theoretical predictions (Ritz et al., 2007). The grafting of ATRP initiators has also been tried for synthesis of block polymer brushes and modification of substrates. In this direction, a new ATRP initiator, bromoisobutyrateundecyl phosphonic acid has been synthesized and grafted as a film on a mechanically polished stainless steel (AS1304) substrate. The use of the corresponding initiator-modified substrates is polymethylmethacrylate and diblock polystyrene-*b*-polymethylmethacrylate in synthesis of ATRP polymer and copolymer brushes of polystyrene (Minet et al., 2009). Recently, the synthesis of designed tri-block copolymers have been reported using different polyurethane (PU) based macroinitiator through ATRP (Tharanikkarasu et al., 2008; Verma & Tharanikkarasu, 2008a; Verma & Tharanikkarasu, 2009) and reverse ATRP (Verma & Tharanikkarasu, 2008b) strategies. However, tri-block copolymers with broad molecular weight distribution (MWD) values were obtained. To get rid of this problem, a novel tertiary bromine (-C(CH<sub>3</sub>)<sub>2</sub>Br)-terminated PU was prepared and used successfully in polymerization of methylmethacrylic acid (Verma & Tharanikkarasu, 2008c).

### Graft polymers

In recent years, graft modification of polymers and its derivatives have attracted considerable attention in the development of polymeric membranes, polymeric coatings and materials for drug delivery (Nishio 2006). Various

approaches of grafting have been developed, such as free radical polymerization (FRP) (Pourjavadi & Mahdavinia, 2005; Gupta & Khekar, 2006), ring-opening polymerization (ROP) (Teramoto et al., 2004), NMP (Daly et al., 2001), RAFT (Roy et al., 2005), and ATRP (Tang et al., 2007; Ostmark et al., 2007). Among these techniques, ATRP has been extensively used for graft copolymerization of vinyl monomers into polymers or polymeric derivatives in a living/controllable way because of its easy manipulation, ability to control molecular weight, structure and applicability to a wide range of monomers. The graft copolymerization can be of two types either grafting from or grafting to processes (Minko 2008). From the last two decades, various ATRP macroinitiators have been synthesized for graft polymerization for example, polystyrene based ATRP macroinitiators have been synthesized for graft polymerization of polystyrene. Low-molecular-weight polystyrene (PS) and poly(4-methyl styrene) [P(4-MS)], have been used as starting materials and they were functionalized by introducing the 2-bromopropionyloxy or 2-bromoisobutyryloxy functionality in the benzene rings of PS, and bromination on the methyl groups of poly(4-methyl styrene) [P(4-MS)] (Janata et al., 2001). In another study, graft copolymer hydroxyethylcellulose-graft-poly(*N,N* dimethylacrylamide) (HEC-g-PDMA) has been synthesized by using ATRP. First, O-(2-hydroxyethyl) cellulose (HEC)-Br macroinitiator was synthesized by reacting HEC with 2-bromoisobutyryl bromide. Then *N,N*-dimethylacrylamide (DMA) was grafted from the HEC-Br macroinitiator in the presence of a catalyst and macrocyclohexaamine (Me<sub>6</sub>(14) aneN<sub>4</sub>) as a ligand. HEC-g-PDMA with well-defined structures was applied as coatings for the separation of basic proteins. The results of the HEC-g-PDMA coating in protein separation have demonstrated the suitability of coating in electrophoretic protein separations (Yang et al., 2010).

Various ATRP initiators have been tried for controlled graft polymerization over the surface for immobilization of enzymes. In one of the study, glucose oxidase enzyme was immobilized over silicon surface by using 4-vinylbenzylchloride as an ATRP initiator. In the reaction, the covalent attachment (Si-C bonding) of 4-vinylbenzyl chloride (VBC) was done on the hydrogenterminated Si (111) surface (Si-H surface) via radical induced hydrosilylation and the resultant, well-defined poly(glycidyl methacrylate) [P(GMA)] brushes were covalently tethered onto silicon surfaces [Si-g-P(GMA) hybrids]. Moreover, P(GMA) is a well-known surface linker and spacer for biomolecules

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(Grano et al., 2004). Therefore, the epoxide groups of grafted P(GMA) brushes were utilized in direct coupling of glucose oxidase (GOD) (Xu et al., 2005). In another study, polyacrylamide has been grafted over silicon surfaces to prevent microbial adhesion to solid surfaces. The polyacrylamide (PAAm) brushes have grown on silicon surface by ATRP using a three-step reaction procedure consisting of immobilization of a coupling agent 3-aminopropyltriethoxysilane, anchoring of an ATRP initiator 4-(chloromethyl)benzoyl chloride, and polymerization of acrylamide (Cringus-Fundeanu et al., 2007). The intelligent dual responsive surface has also been synthesized by grafting of polyvinylpyrrolidone (PVP), poly-*N*-isopropylacrylamide (PNiPAAm), PVP-*b*-PNiPAAm, and PNiPAAm-*b*-PVP, respectively from a cellulose fiber substrate (filter paper) using surface-initiated ATRP. The accessible hydroxyl groups on the surface of cellulose substrates were thereafter converted into ATRP initiators by reaction with 2-chloropropionyl chloride or 2-bromoisobutyryl bromide (Lindqvist et al., 2008). In a similar study, thermo-responsive membrane based microporous polypropylene membrane (MPPM) has been synthesized. In this study, comb-like poly(*N*-isopropylacrylamide) (PNiPAAm) was grafted onto MPPM surface using surface-initiated ATRP. Hydroxyl groups were first introduced onto membrane surfaces by ultraviolet-induced graft polymerization of 2-hydroxyethylmethacrylate. Then, initiators were coupled through reaction between tethered hydroxyl groups and 2-bromoisobutyryl bromide, which initiated the reaction of NiPAAm (Wan et al., 2009).

**Star polymers**

In recent years, with the advent of controlled radical polymerization techniques especially ATRP has greatly expanded the possibilities of synthesizing well-defined polystyrene (PS) and various poly(methacrylate) stars with an exact number of arms by core-first approach (Kamigaito et al., 2001). Most of the employed initiators are based on multifunctional inorganic or organic compounds with halogen or hydroxyl groups, where latter is initially converted to chloro- or bromoesters. Three-arm stars have also been prepared by ATRP employing polyols (Ueda et al., 1998b; Matyjaszewski et al., 1999) or phenols (Haddleton & Waterson, 1999). More recently, a phenol is also shown to be formed, which utilized the basis for three-arm star molecular brushes with poly(*N*-butyl acrylate) side chains (Matyjaszewski et al., 2003). Furthermore, amphiphilic

poly(methacrylate) three-arm star block copolymers (Narainen et al., 2002) based on phenols and miktoarm polystyrene (PS) (Francis et al., 2002) have been fabricated by ATRP. Similarly, four-arm star polymers have also been constructed employing either polyol- or cyclosiloxane-based initiators or calyx (4, 6, 8) arenes (Angot et al., 2000) and RAFT polymerization by using polyols or tetrabromomethyl benzene (Mayadunne et al., 2003). Five-arm PS and poly(methylmethacrylate) (PMMA) have also been synthesized from a glucose-derived initiator (Haddleton et al., 1999). First time, six-arm PS was prepared in 1995 by ATRP, which was based on hexakis (chloromethyl) benzene initiator (Wang et al., 1995). Later cyclotriphosphazene (Francis et al., 2002) calix(6)arene (Ueda et al., 1998a), or hexahydroxytriphenylene (Feng & Pan, 2001) have been employed for both PS as well as poly(methacrylates) stars. Furthermore, octafunctional calyx (8) arene has been utilized in the construction of PS (Angot et al., 1998) and poly(methacrylates) stars. In this series, eight-arm PMMA stars have also been prepared from a sucrose-based initiator (Haddleton et al., 2000). Moreover, twelve-arm PMMA stars have been synthesized by using ATRP initiated by a dendrimer-like twelve arm ester initiator (Heise et al., 2001). Additionally, various aromatic multisulfonyl chloride initiators (Percec et al., 2000) or metal complexes with polymeric ligands (Fraser & Smith, 2000) have been employed for synthesis of numerous star polymers. In this direction, multifunctional macroinitiators (MI) have been synthesized for ATRP reaction in synthesis of star polymers based on styrene and methyl, *n*-butyl, and *n*-hexyl acrylate monomers. In these reactions, MIs based on ditrimethylolpropane (diTMP) (4-OH groups) and dipentaerythritol (diPENTA) (6-OH groups) were converted into corresponding bromoesters. Additionally, a highly branched polyether poly(3-ethyl-3-hydroxymethyloxetane) (PEOX) (about 12-OH groups) has been employed as an ATRP initiator in synthesis of block polymers (Jankova et al., 2005).

Furthermore, two types of three-arm star mesogen-jacketed crystal polymers 2, 5-bis[(4-methoxyphenyl)oxycarbonyl]styrene (MPCS) with different core (hard core and soft core) have been synthesized by using two different trifunctional initiators 1, 3, 5-(20-bromo-20-methylpropionato) benzene and 1, 1, 1-tris(2-bromoisobutyryloxymethyl) propane, respectively (Wang et al., 2005). Recently, Gao and his group have developed a new method for synthesis of star polymers with high

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molecular weight, yield and low polydispersity via copolymerization of linear macromonomers with a divinyl cross-linker using low molar mass ATRP initiators (MM method) (Gao et al., 2006). In this method, the number of initiating sites (derived from the initiator) and arms (derived from macromonomers) per star molecules is independently controlled. A low molar ratio of initiator to macromonomers usually decreases the number of initiating sites in star core, which reduce the possibility of star-star coupling reactions and decreases star polydispersity. Moreover, macromonomers, initiators, and cross-linkers can be added in several steps, so that flexibility of star polymer synthesis can be increased. An extra advantage of this new macromonomers method is that additional functional groups can be easily introduced into star core via functional initiators. The synthesis and application of functional ATRP initiators are much easier and more efficient than the displacement of embedded alkyl halide chain ends in the preformed star core. The poly *n*-butylacrylate-polydivinyl benzene (pyrene) star polymers have also been synthesized using a pyrene containing ATRP initiator (Py-Br) to initiate the copolymerization of polyBA macromonomers and divinylbenzene (DVB) (Gao & Matyjaszewski, 2007). In this direction, a series of 10- and 20-arm star like block copolymers containing inner soft poly(*N*-butylacrylate) (PBA) block and outer hard poly(methylmethacrylate) (PMMA) blocks were synthesized by ATRP. The small macroinitiators have also been designed for synthesis of poly(2-bromoisobutyryloxyethyl acrylate) (PBiBEA) star polymer with high degree of polymerization by ATRP of trimethylsilyloxyethyl acrylate (HEA-TMS) and subsequently followed by esterification. In some of the reports of star polymers, the partial star coupling reaction has been observed during star extension with polymeric blocks, and in this case the coupling can be increased with increasing number of arms and arm length (Nese et al., 2010).

#### End functionalized polymers

Polymer peptide bioconjugates are of great interest because of their potential applications in biotechnology, industry and in medical fields (Lutz & Borner, 2008). They are commonly synthesized by ring-opening of an *N*-carboxyanhydride followed by polymerization using polypeptide as a macroinitiator (Knoop et al., 2008). In other ways, amino group functionalized polymer chain can also be used for ring opening of *N*-carboxy anhydride in synthesis of polymer-peptide conjugates (Dimitrov & Schlaad, 2003;

Sinaga et al., 2007). Recent literatures have reported the use of living radical polymerization techniques such as ATRP to synthesize polymer-peptide conjugates (Rettig et al., 2004; Becker et al., 2005). ATRP is widely used for the preparation of well-defined polymers and their bioconjugation with peptide/protein. Moreover, ATRP is robust and can be applied with a wide range of monomers.

It is well known that the choice of initiator has an important role in ATRP. The most commonly used initiators are those with an  $\alpha$ -haloester group with the halogen atom, where homolytic fission takes place in the presence of a catalyst (most often a copper (I) halide). The strength of the CAX (X  $\frac{1}{4}$  halogen) bond in initiator systems should be in optimum range because in general, homolytic fission will not occur if the bond strength is too high and hence probability of polymerization is decreased. While, if the bond strength is too low, then high concentration of free-radicals is produced, this leads to uncontrolled polymerization resulting in premature termination as well as high polydispersity of polymers. The ester unit on such initiators usually carry this optimum range of bond strength with different functional groups, which is often exploited in synthesis of block copolymers with preparation of a polymeric initiator such as properly esterified methoxy poly(ethylene oxide) (Liu et al., 2002; Adams et al., 2006). Despite the ubiquity of ester based initiators in ATRP, the use of amide based initiators are rare, although the use of such amide-based initiators would greatly enhance the synthesis of novel polymeric materials that can be prepared by ATRP, for example peptide-polymer conjugates from peptide-based initiators. A number of successful polymerizations with amide initiators have been reported using ATRP (Couet et al., 2006a, 2006b; Loschonsky et al., 2008) but few literatures have proved the inability of amide initiator in ATRP reaction conditions, e.g., limited conversion in the polymerization of *N,N*-dimethylacrylamide and loss of catalyst during the reaction (Teodorescu & Matyjaszewski, 2000; Neugebauer & Matyjaszewski, 2003). Additionally, amide based initiators have been reported to have poor efficiency, which can lead to less monomer conversions and produce polymers with high polydispersity index (Li et al., 2005). Furthermore, copper catalysts with amide based initiators do not efficiently work in inducing radical addition, which is attributed to complications between the amide and copper (Senoo et al., 1999). Despite the above mentioned limitations, there are recent reports, where amide based initiators originated from amino acids work well in ATRP initiation (Teodorescu &

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Matyjaszewski, 1999). These amino acid based initiators are synthesized polymers with low polydispersity index, but higher molecular weights than expected one, which implies that significant chain termination occurs during the initiation step. There are also examples of functionalized peptides or protein based initiators, which can successfully utilize the polymerization of monomers in ATRP (Lele et al., 2005). In one of the example, oligo(phenylalanine)-based amide ATRP initiators used an efficient polymerization of *N*, *N*-diethylaminoethylmethacrylate (DEA) and tert-butylmethacrylate and results have been compared to a commonly used ester-based initiator, ethyl-2-bromoisobutyrate (E2BB) (Adams & Young, 2008). The copolymer synthesized through oligo(phenylalanine)-based initiators have low dispersity index than polymers with ester based initiators.

A number of ATRP initiators have been synthesized with protein-reactive groups resulting in R-functional polymers (Heredia & Maynard, 2007), like pyridyl disulfide (Bontempo et al., 2004), and maleimide chemical moiety (Mantovani et al., 2005). Moreover, biotinylated ATRP initiators (Qi et al., 2004; Vazquez-Dorbatt & Maynard, 2006) synthesize end functionalized polymers, which can be conjugated to streptavidin protein. In another example of peptide-polymer conjugates, aminoacids are first modified with ATRP initiators for the copolymerization of styrenes and methacrylates monomers. The Fmoc-4-(1-chloroethyl)-phenylalanine was synthesized in four steps from Fmoc-tyrosine (Elemans et al., 2003). The HATU-mediated amidation with glycine-OMe has led to the formation of dipeptide (Velonia et al., 2002). The initiator was effective for Cu(I)/bipyridine catalyst for bulk polymerization of styrene monomer (Broyer et al., 2008).

**CONCLUSION**

ATRP based polymer-protein bio-conjugation has also shown several advantageous features over conventional bioconjugation reactions, such as regulated modification of native proteins, synthesis of well-defined molecular weight conjugates with retention of high residual activity as well as ease of purification. Novel monomers have also been designed and used in ATRP for modification of proteins with unique properties that are not possible with simple conjugation reactions. These advantages make protein-initiated ATRP a promising and an attractive alternative technique for future research to develop new and well-defined polymer-protein bioconjugates.

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