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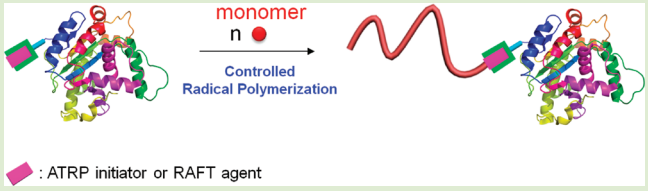
Proteins as Initiators of Controlled Radical Polymerization: Grafting-from via ATRP and RAFT

Brent S. Sumerlin*

Department of Chemistry and Center for Drug Discovery, Design, and Delivery, Southern Methodist University, Dallas, Texas 75275-0314, United States

ABSTRACT: Many recent developments in polymer chemistry have advanced the synthesis of materials in which synthetic polymers are immobilized to biological (macro)-molecules to enhance the solubility, stability, activity, or therapeutic utility of the biological entity. In particular, the versatility and robust nature of controlled radical polymerization (CRP) has enabled access to a diverse family of new polymer bioconjugates. While nitroxide-mediated, atom transfer radical (ATRP), and reversible addition–fragmentation chain transfer (RAFT) polymerizations have all proven useful for the preparation of well-defined end-functional polymers capable of being efficiently conjugated to biological molecules, ATRP and RAFT have proven especially proficient for the synthesis of conjugates by direct polymerization of vinyl monomers from biological components functionalized to contain a group capable of initiating chain growth. This Viewpoint highlights several recent advances that have relied on grafting-from by CRP, with particular attention devoted to a recent report that seeks to facilitate the process of grafting-from proteins via ATRP under biologically relevant conditions.



Advances in synthetic polymer chemistry over the last fifteen years have enabled access to a wide variety of well-defined functional polymers of controlled molecular weight and architecture. Recently, there has been considerable interest in using these previously inaccessible polymers to augment the evolved sophistication of biological macromolecules. Conjugation of synthetic polymers to proteins, peptides, polysaccharides, and nucleic acids has proven to be a viable means of enhancing the activity, therapeutic utility, solubility, and stability of the biological molecule being modified.

Of particular interest in the field of polymer bioconjugates, polymer–protein/peptide conjugates have proven utility in medicine, sensing, and enzymatic catalysis, among others. For applications in vivo, the polymer is generally intended to provide enhanced bioactivity or passive stabilization of the protein or peptide to allow increased blood circulation time, improved targeting, endosomal release, and so on. While the most common polymer employed in this respect has been poly(ethylene glycol) (PEG) or its derivatives, largely because of reduced toxicity and immunogenicity and increased biocompatibility, recent attention has been dedicated to alternative methods of "PEGylation" by the polymerization of PEG or oligo(ethylene glycol) (OEG) vinyl macromonomers.[1,2] There has also been growing interest in considering other water-soluble, hydrophobic, or stimuli-responsive synthetic polymers for the modification of proteins.[3] Providing many of the benefits of PEG, these polymers may simultaneously provide sites for subsequent attachment of cofactors, imaging reagents, and targeting ligands while also leading to responsive assembly or activity modulation of the attached protein.

The preparation of conjugates from vinyl monomers has been significantly aided by developments in the field of controlled radical polymerization (CRP). The range of monomers, solvents, and reaction conditions that most CRP methods can tolerate has allowed the synthesis of a variety of previously inaccessible polymer–protein conjugates. While there are comprehensive reviews describing the role of CRP during the synthesis of polymer bioconjugates,[4] this Viewpoint is meant to highlight a handful of recent advances in the area. Following a brief overview of the basic synthetic strategies commonly employed to prepare polymer bioconjugates, particular emphasis will be given to polymer–protein conjugates prepared by CRP from proteins modified with a moiety capable of chain initiation.

Coupling polymers to reactive sites on proteins, commonly called the "grafting-to" approach, allows the synthesis of conjugates from a library of preformed polymers (Scheme 1).[5] Typically, this strategy involves the covalent immobilization of end-functional polymers to complementary functional groups present on native or activated amino acid residues. CRP is particularly effective for the synthesis of end-functional polymers, and atom transfer radical polymerization,[6] reversible addition–fragmentation chain transfer (RAFT) polymerization,[7] and nitroxide-mediated polymerization (NMP)[8] have all been utilized to prepare a range of polymers that have been conjugated to therapeutic and enzymatic proteins. While attractive because of its modularity, the grafting-to approach often requires an excess of functional polymer to overcome

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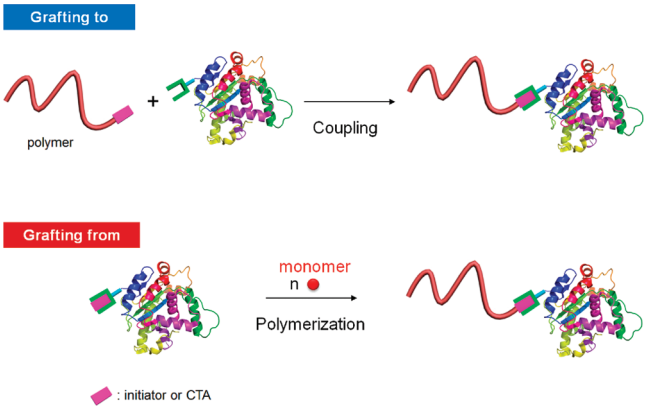
Scheme 1. General Synthetic Strategies to Polymer–Protein Conjugates

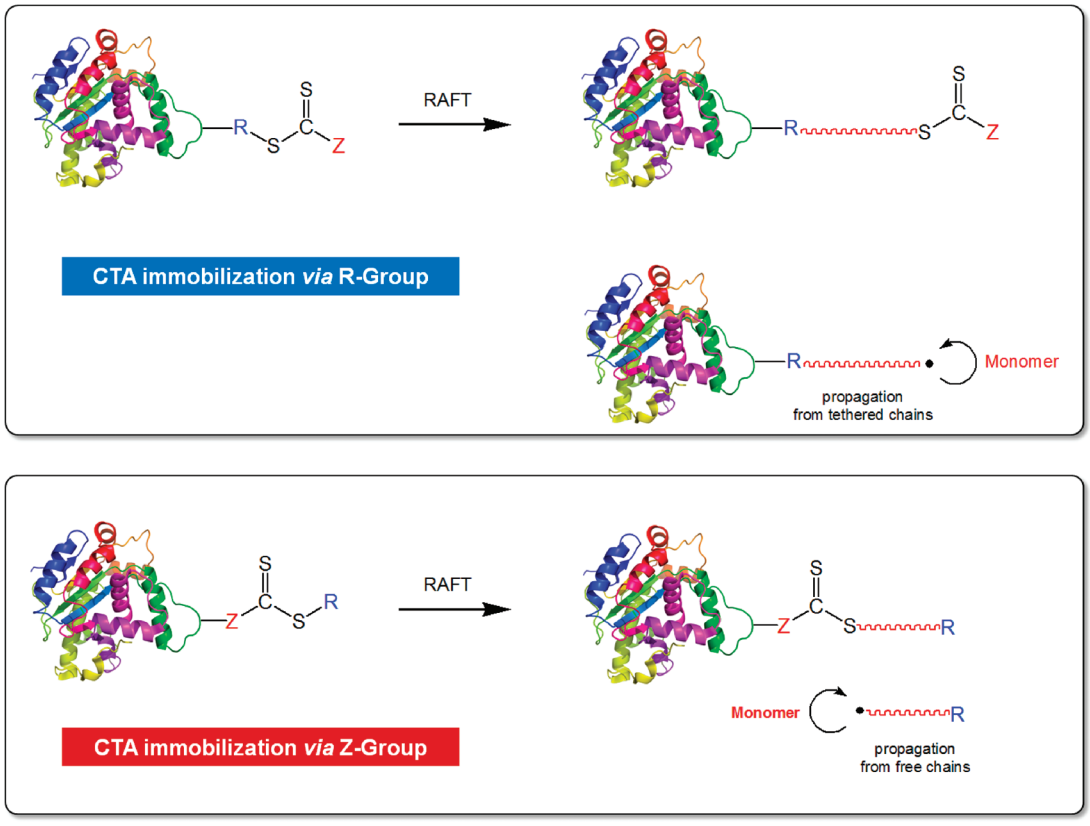


steric limitations and the inherent low concentration of functional groups involved in the conjugation reaction. Separation of unreacted protein or polymer can be challenging, and the immobilization of high molecular weight polymer can be especially problematic.

Fortunately, the inherent functional group tolerance and aqueous amenability of radical polymerizations allows the conjugation process to be accomplished in situ by initiating polymerization directly from the surface of an activated protein (Scheme 1). This "grafting-from" approach offers several benefits. Purification is simplified because unreacted monomer is readily removed from the final polymer–protein conjugate. Additionally, because many of the problems associated with the sterics of coupling are avoided, this strategy is particularly well suited to the preparation of conjugates with high molecular weight homopolymers or block copolymers.

Of the various CRP methods, RAFT and ATRP have proven most effective for the synthesis of polymer–protein conjugates by the grafting-from approach. The ease with which RAFT can be conducted in water in the presence of the diverse array of functional groups present on proteins has allowed it to become a highly useful method for the synthesis of homopolymer and block copolymer conjugates.[9] Proteins modified by grafting-from to yield chains of poly(monomethoxy poly(ethylene glycol)-(meth)acrylate) (PPEG(M)A),[10,11] poly(*N*-isopropylacrylamide) (PNIPAM),[12,13] and poly(*N*,*N*-dimethylacrylamide) (PDMA),[14] among others, have been prepared by RAFT polymerization from proteins modified with suitable RAFT chain transfer agents. To achieve the characteristics associated with the grafting-from strategy, the RAFT agent (Z-C(=S)S-R) is immobilized to the biological substrate via its R-group such that the thiocarbonylthio moiety is distal to the protein and readily accessible for chain transfer with propagating chains in solution, a key step of the RAFT mechanisms responsible for molecular weight control.[13,15] Moreover, this "R-group approach" leads to the relatively labile thiocarbonylthio group residing on the free end of the immobilized polymer such that it is not responsible for the conjugation linkage. Therefore, the conjugates prepared in this way may demonstrate increased stability and the potential for transformation into thiol groups for subsequent surface immobilization, labeling, or chain extension. For example, we recently reported methods for the synthesis of block copolymer–protein conjugates by two

Scheme 2. R-Group (top) and Z-Group (bottom) Strategies for Preparing Polymer–Protein Conjugates by Reversible Addition–Fragmentation Chain Transfer (RAFT) Polymerization



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consecutive grafting-from polymerizations.[14] Access to the terminal thiocarbonylthio group retained during the initial polymerization of NIPAM that yielded the PNIPAM−protein conjugates allowed subsequent chain extension with *N*,*N*-dimethylacrylamide. Facilitated access to the end group also allows the preparation of conjugates with well-defined high molecular weight chains, because intermolecular transfer during the RAFT main equilibrium occurs by reaction of the propagating chains in solution with the sterically accessible thiocarbonylthio group on the free end of the immobilized polymer.

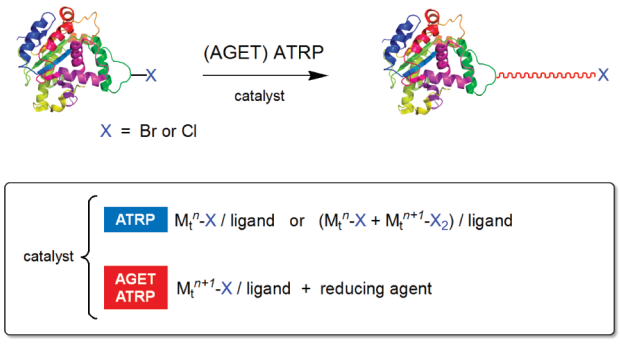
An alternative approach for the formation of well-defined polymer−protein conjugates involves immobilization of the RAFT agent to the protein via its Z-group (Scheme 2). While not strictly a grafting-from process, this highly efficient "transfer-to" method was the first reported to lead to polymer−protein conjugates directly during RAFT polymerization.[10,12] A benefit of this approach is that having the polymer linked to the protein via its Z-group ensures that only dormant "living" chains are conjugated, because all termination products remain in solution. Additionally, to allow separate characterization of the polymer or for in vivo applications in which triggered chain cleavage is beneficial, having the polymer and protein linked via the relatively labile thiocarbonylthio group can be advantageous.

Despite the recent success of RAFT, the first CRP method used for grafting well-defined polymers from proteins was ATRP. Early reports by Russell, Matyjaszewski et al.,[16] Maynard et al.,[17] and Haddleton et al.[2] demonstrated that ATRP could be employed to efficiently prepare a variety of functional conjugates of model proteins with PPEGMA and PNIPAM. The application of ATRP for the preparation of polymer bioconjugates has flourished, with many other reports describing polymerization from proteins or other biological molecules modified with activated alkyl halide initiators to yield giant amphiphiles,[18] virus-based polymer−protein nanoparticles,[19] and polymer−peptide[20] conjugates.

ATRP is well-suited for the preparation of bioconjugates by a grafting-from approach. The applicability to a wide range of monomers commonly polymerized in aqueous environments, most notably (meth)acrylates and (meth)acrylamides, allows access to a diverse set of conjugates. In particular, the readily controlled polymerization of PEG-based macromonomers and temperature-responsive PNIPAM affords conjugates that can be envisioned to have therapeutic, diagnostic, and enzymatic utility. However, despite the success of ATRP for the synthesis of polymer−protein/peptide conjugates, the reliance on transition metal catalysts, most often copper, has often been cited as a potential concern. While the extent of the potential complications of trace metals is debatable, at a minimum the perception of these complications may limit the more widespread adoption of ATRP for the preparation of biomaterials. However, recently reported variations of ATRP, such as activators generated by electron transfer (AGET),[21] initiators for continuous activator regeneration (ICAR),[22] and activators regenerated by electron transfer (ARGET)[22,23] have proven particularly useful at addressing the potential complications of residual catalyst in polymeric products (Scheme 3).[24]

Perhaps of equal concern during the synthesis of bioconjugates by grafting-from via ATRP are the challenges of employing Cu(I) catalysts in protic media.[25] In addition to hydrolysis of the alkyl halide initiators and chain ends, without proper precautions, aqueous ATRP is complicated by a variety of factors related to catalyst stability. Among these, halide dissociation from the Cu(II) deactivating species can occur and be immediately followed by competitive coordination of solvent to the catalyst. Additionally, aqueous media can lead to destabilization of many Cu/ligand complexes and/or rapid disproportionation or oxidation of the Cu(I) activating species.

Scheme 3. Polymer-Protein Conjugation by Grafting-from via Atom Transfer Radical Polymerization (ATRP) or Activators Generated by Electron Transfer ATRP (AGET ATRP)



AGET ATRP has been recently applied to the synthesis of polymer−protein conjugates by polymerization of PEGMA macromonomers from initiator-functionalized recombinant human growth hormone[26] and trypsin.[11] A benefit of AGET ATRP is that the Cu(I) activator is formed in situ from an oxidatively stable Cu(II) complex. In these cases, the polymer-modified proteins were shown to have enhanced stability against denaturation and proteolysis, presumably due to the presence of the immobilized polymer. Additionally, efficient conjugation did not come at the expense of protein activity. As these recent examples suggest, many of the challenges of aqueous ATRP bioconjugations can be addressed by proper selection of the polymerization conditions.

In this respect, it would be advantageous to have a systematic set of guidelines to consult when selecting the catalyst system and reaction setup for use in aqueous polymerizations that involve grafting from proteins by ATRP. A recent report by Matyjaszewski and co-workers provides considerable insight into the appropriate selection of reaction conditions to allow efficient and well-controlled aqueous ATRP from proteins modified with an alkyl halide initiator.[27] Polymerizations of monomethyl ether oligo(ethylene glycol) methacrylate (OEGMA) were conducted under biologically relevant conditions (i.e., in aqueous media, near-ambient temperature, with low organic content, and in the presence of a catalyst that would not significantly bind with or denature model proteins; Figure 1). This systematic investigation of polymerization conditions demonstrated that ATRP of OEGMA macromonomers from bovine serum albumin (BSA) modified to contain up to 30 bromoisobutyryl initiating groups could be readily accomplished by both normal and AGET ATRP. By using an initiating moiety that was susceptible to basic hydrolysis after polymerization, the resulting polymers could be readily cleaved and characterized independently. It was observed that grafting from BSA by ATRP could be particularly well controlled in a variety of aqueous solvent systems. During normal ATRP in H_2O, polymerization control was enhanced by employing CuCl instead of CuBr, a high Cu(II)/Cu(I) ratio, and a ligand that limited the activity of the catalyst to prevent

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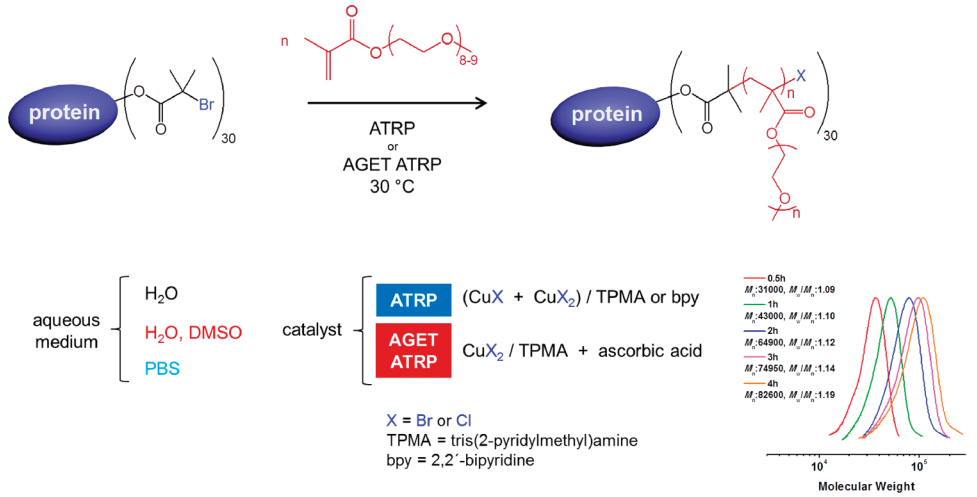

Figure 1. Grafting-from via aqueous ATRP and AGET ATRP to yield poly(monomethyl ether oligo(ethylene glycol) methacrylate)–bovine serum albumin conjugates. A variety of aqueous solvents and catalyst systems were considered. Chain cleavage after polymerization allowed molecular weight analysis by size exclusion chromatography.[27]

significant termination early in the polymerization, methods that had previously been reported to allow control of aqueous ATRP.[25]

The added benefit of using an oxidatively stable Cu(II) catalyst that can tolerate limited amounts of oxygen and can be reduced to Cu(I) in situ led the authors to also consider AGET ATRP for the bioconjugation polymerizations.[27] In this case, conversion of Cu(II) to Cu(I) was accomplished by ascorbic acid, a rather innocuous reducing agent that helped to maintain the biological relevance of the polymerization conditions. While adding the reducing agent in one step at the beginning of the polymerization led to polymerizations that stopped at low conversion, significantly enhanced control was achieved by slow addition over the course of the polymerization. The evolution of molecular weight with conversion and narrow molecular weight distributions of the grafted poly(OEGMA) chains were consistent with the typically well-controlled polymers formed by ATRP from low molecular weight initiators. For example, molecular weights of up to M_n = 83 kg/mol (M_w/M_n < 1.2) were obtained in 4 h at 30 °C.

Polymerizations in phosphate buffered saline (PBS) and H_2O/DMSO mixtures were also considered. PBS is a commonly used solvent for proteins. Despite the potential complications of using copper halide catalysts in PBS, well-controlled polymerizations were observed during AGET ATRP with CuBr/$CuBr_2$ catalyst systems. Similar success was obtained in H_2O/DMSO mixtures, suggesting the approach can be extended to the polymerizations of hydrophobic monomers that require organic cosolvents for solubility.

Many relatively recent developments in CRP methodologies have significantly advanced the field of polymer bioconjugation. In particular, both RAFT and ATRP have proven to be especially well suited for the synthesis of polymer–protein conjugates by the grafting-from approach. In addition to selecting the specific CRP method to employ, it is important to carefully consider the polymerization conditions that will lead to well-defined polymers while maintaining the structure, stability, and activity of the protein being modified. In particular, given the challenges associated with conducting ATRP in aqueous media, the polymerization guidelines reported by Matyjaszewski and co-workers will likely prove to be especially valuable for those in the bioconjugation field. It is particularly important that the studies focused on the preparation of polymer–protein conjugates under conditions that were biologically relevant and protein-friendly, because the ultimate success of ATRP for the preparation of conjugates with many other types of biological macromolecules will likely rely on adherence to many of these basic principles. In this respect, the recent Letter is a valuable resource for both newcomers and experts in the field of bioconjugation and will likely enable the preparation of a wide variety of novel polymer–protein biomaterials.

AUTHOR INFORMATION

Corresponding Author

*E-mail: bsumerlin@smu.edu.

Notes

The authors declare no competing financial interest.

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