Published in final edited form as:

ACS Macro Lett. 2018 ; 7(10): 1248–1253. doi:10.1021/acsmacrolett.8b00609.

Synthesis of Polymer Bioconjugates via Photoinduced Atom Transfer Radical Polymerization under Blue Light Irradiation

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Abstract

A rapid blue-light-induced atom transfer radical polymerization (ATRP) was conducted in a biologically friendly environment. Well-controlled polymerization of oligo(ethylene oxide) methyl ether methacrylate (OEOMA) was successfully performed in aqueous media (1X PBS) under irradiation by blue LED strips. With 10.0 mW/cm² intensity output at 450 nm, >90% conversion was achieved in 2 h in the presence of a system comprising glucose, glucose oxidase, and sodium pyruvate. Poly-(OEOMA) was synthesized with predetermined M_n and low dispersities using low ppm of Cu catalysts. Importantly, secondary structures of proteins, as analyzed by circular dichroism (CD), were preserved under blue-light irradiation due to its lower energy output. The aqueous blue-light ATRP technique was applied to biological systems by synthesizing well-defined protein–polymer and DNA–polymer hybrids by the “grafting-from” method.

Graphical Abstract

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacro-lett.8b00609. Experimental procedures, results and discussion, and additional references (PDF)

The authors declare no competing financial interest.

Atom transfer radical polymerization (ATRP) is one of the most attractive controlled radical polymerization techniques that has been widely employed to prepare polymers with precise molecular weight, uniform chain length, predefined topology, and high end group functionality.¹ Traditional ATRP required high catalyst concentration to maintain activity throughout the polymerization process. However, in recent years, several new ATRP techniques were developed by continuous regeneration of active Cu(I) species with various external stimuli. These techniques include activators generated by electron-transfer (AGET) ATRP;² activators regenerated by electron-transfer (ARGET) ATRP;³ initiators for continuous activator regeneration (ICAR) ATRP;⁴ supplemental activators and reducing agent (SARA) ATRP;⁵ electrochemically mediated ATRP (eATRP);⁶ photoinduced (Photo-) ATRP;⁷ and mechanically induced (Mechano-) ATRP.⁸ These newly developed techniques allow the utilization of ppm level of Cu catalysts and various choices of external stimuli and solvents based on the reactants' nature.⁹

ATRP has been conducted under various conditions including homogeneous (solution), heterogeneous (suspension, emulsion), and bulk systems.¹⁰ In a homogeneous ATRP system, organic solvents are the most often used media, but they can be volatile and potentially hazardous. Thus, water is a desirable replacement due to its safe, economic, and environmentally friendly properties and because it allows direct synthesis of water-soluble polymers. Another significant advantage of using water as solvent is the ability to graft from biomolecules such as proteins, DNA/RNAs, and even bacteria and cells. Biomolecules can preserve their tertiary structures during grafting-from under biologically relevant conditions such as ARGET and ICAR ATRP due to the low catalyst loading and benign polymerization conditions.¹²

Besides ARGET and ICAR ATRP, photo-ATRP is also a powerful option for performing ATRP in aqueous media. In ARGET or ICAR ATRP protocols, new reactants are introduced into the systems, i.e., reducing agents or conventional radical initiators, which might require further purification steps. Photo-ATRP, however, requires only a slight excess of ligands (electron donors) and proper light irradiation. Recently, our group reported photo-ATRP in aqueous media using visible (violet) light for synthesis of well-defined polymers.¹³ Sumerlin and co-workers reported successful protein—polymer conjugate synthesis by photoinduced electron transfer reversible addition—fragmentation chain transfer (PET-RAFT) polymerization, in which eosin Y and blue light were utilized.¹⁴ However, the synthesis of protein—polymer conjugates via photo-ATRP has not yet been reported. The major drawback of applying photo-ATRP to polymer bioconjugate synthesis is that prolonged irradiation with ultraviolet or violet light can damage the disulfide linkages in a protein's secondary structures.¹⁵ Thus, light sources with weaker energy output would be ideal.

Herein, we report the first photo-ATRP using blue light in aqueous media to synthesize polymer bioconjugates. With commercially available, inexpensive, and easily applicable LED strips, ATRP of OEOMA₅₀₀ was carried out under blue-light irradiation (450 nm, 10.0 mW/cm²) in water or 1X phosphate buffer saline (PBS) with ppm level of the Cu^{II}/tris(2-pyridylmethyl)amine (TPMA) complex. Approximately 90% monomer conversion was

reached in 5 h, while the polymer retained a precise match of M_n with theoretical values and low dispersities.

To further investigate the biorelevant conditions, instead of bubbling inert gas (N_2), the deoxygenation system comprising glucose, glucose oxidase, and sodium pyruvate (Glu + GOx + SP)^{12b} was applied to the blue-light photo-ATRP system and exhibited excellent performance. With the addition of Glu + GOx + SP, the rate of polymerization significantly increased (>95% conversion in 2 h), and polymers with even lower dispersities were obtained. Meanwhile, the successful use of GOx indicated that the environment was biocompatible and harmless for proteins or other biomolecules. Eventually, well-defined protein— and DNA—polymer conjugates were efficiently synthesized and analyzed by dynamic light scattering (DLS), while the synthetic polymers were characterized by gel permeation chromatography (GPC) after cleavage by 5% sodium hydroxide solution.

Proteins, especially enzymes, are among the most important components of biological systems. Most proteins in their native states exhibit precise unique quaternary, tertiary, and secondary structures to exhibit their exceptional activities. However, when exposed to strong acids or bases, concentrated salts, organic solvents, or physical extremes such as heat or radiation, the secondary and tertiary structures of proteins can be lost and result in irreversible denaturation. Strong, high energy ionizing radiations such as γ -ray, X-ray, and short-wave ultraviolet light are extremely harmful to cells, and even medium wavelength irradiation might induce mutations to DNA¹⁶ and cause denaturation of proteins.¹⁷ Therefore, to employ proteins in photo-ATRP, it is essential to choose a biologically tolerant light source.

To visualize how intensity of different light sources could affect proteins' secondary structures, circular dichroism (CD) measurements were carried out with bovine serum albumin (BSA) and glucose oxidase (GOx) before and after 2 h of irradiation at room temperature. Three light sources were applied: UV nail-curing lamp (365 nm, 0.9 mW/cm²), violet LED strip (392 nm, 3.5 mW/cm²), and blue LED strip (450 nm, 10.0 mW/cm²). Comparatively, BSA is a more robust protein and was able to withstand all types of irradiation without showing a significant CD change (Figure 1A). GOx, however, is relatively more delicate when exposed to long-time irradiations. The CD signals of GOx changed quite remarkably from the control group when irradiated by UV and violet light. However, blue light did not cause any spectral change as compared to the control signal (Figure 1B). This set of experiments indicated that blue light was a more suitable source of light for biological systems due to its lower energy output. Although its intensity was the highest, the total damage to protein's secondary structure was the smallest. Thus, blue light could be considered as an optimal source for ATRP photochemical reactions involving protein.

The application of blue light for photo-ATRP was previously reported in organic solvents. Although photo-ATRP was successfully performed in aqueous media by violet light, using blue light in aqueous media has not yet been reported. In this work, photo-ATRP of oligo(ethylene oxide) methyl ether methacrylate (OEOMA, M_n = 500) was conducted using poly(ethylene glycol)₂₀₀₀— α -bromophenylacetate (PEG₂₀₀₀BPA) as a macroinitiator under

blue-light irradiation (450 nm at 10.0 mW/cm²) (Table S1). Starting with DP_T = 200 using 1000 ppm of CuBr₂ (vs monomer), 5-fold excess of TPMA was added as a ligand and also as a reducing mediator since it could efficiently reduce Cu(II) deactivator to Cu(I) activator under photochemical conditions. The resulting oxidized ligand may initiate a small fraction of new chains. Before the irradiation, inert gas (N₂) was purged into the Schlenk flask for 30 min to remove oxygen, while air flow was continuously blown into the lamp in order to prevent temperature increase. 89% monomer conversion was reached after 5 h of irradiation. Excellent control was obtained, and the experimental M_n was in good agreement with the theoretical values with dispersities as low as 1.23 (Figure 2B).

To further simplify the reaction setup and investigate biocompatibility of blue light, a glucose, glucose oxidase, and sodium pyruvate (Glu + GOx + SP) deoxygenating system was utilized for complete oxygen removal, instead of purging N₂ gas. With identical conditions, a shorter reaction time (2 h) was needed to reach 93% conversion. The polymer exhibited good correlation with theoretical values, while its dispersity dropped to 1.13 (Figure 2B). Examination of the kinetics of the above reactions indicated that both reactions gave linear semi logarithmic kinetic plots with a short induction period during the first 30—60 min. The rate of the reaction using the Glu + GOx + SP combination was much higher than the one using N₂ purging, possibly due to the complete removal of oxygen by enzymatic catalysis (Figure 2A).

Subsequently, several ATRP parameters were studied, such as effect of solvent, initiator, DP_T, Cu catalyst concentrations, and ligand equivalence (Table 1). All reactions were performed in 8 mL glass vials with rubber stopper capping, and no further degassing techniques were applied. The initial molar ratio was [OEOMA₅₀₀]₀: [Initiator]₀: [CuBr₂]₀: [TPMA]₀ = 200:1:0.2:1 in 1X PBS at room temperature. ATRP in aqueous media usually requires excess halide salt to maintain good control of molecular weight and dispersity by suppressing the dissociation of the catalytic deactivator complex, which shifts the equilibrium toward a true deactivator complex Br—Cu^{II}Br/TPMA, thus promoting efficient deactivation.^{4,12a} Additionally halogen exchange from bromide to chloride provides higher initiation efficiency and higher stability of chain ends.¹⁹ An analysis of solvent and initiator was compared for 1X PBS (contains 140 mM Cl[−]) vs deionized water + 30 mM sodium bromide and PEG₂₀₀₀BPA vs PEG₂₀₀₀iBBR (Table 1, entries 1—4). These reactions reached 86% conversion in 2 h with good correlation of molecular weights and low dispersities, but reactions conducted in 1X PBS revealed more precisely matching molecular weights with theoretical values and lower dispersities. Various degrees of polymerization were targeted (DP_T) from 100, 200, 400 to 600 (Table 1, entries 4—7); as a result, all of the experiments exhibited excellent consistency of M_n with theoretical value and narrow molecular weight distributions (M_w/M_n 1.23).

Different loadings of Cu catalyst were then tested at DP_T = 400. Initially 1000 ppm of Cu(II) catalytic complex (vs monomer) was utilized but then gradually decreased to 50 ppm (Table 1, entries 7—10). The photo-ATRP provided good control with at least 100 ppm of Cu catalyst but then with a reduced rate of reaction. When 50 ppm of Cu was used, only 8% of conversion was reached in 2 h, and the polymer displayed a much higher value of M_n (75 500) compared to the theoretical value (10 100) as well as molecular weight distribution

($M_w/M_n = 1.54$). The poor control was possibly due to the low deactivator concentration at low ppm of Cu. Meanwhile in photo-ATRP, ligands play an important role as the photoreduction mediators. Thus, an excess of ligand or tertiary amines (i.e., triethylamine) are often added to ensure sufficient regeneration of Cu(I) active catalytic species. Previous studies showed that at least 4X ligand vs CuBr₂ was sufficient for good control of photo-ATRP.¹³ Therefore, with only 1.2X ligand vs CuBr₂, the conversion stopped at 55% in 2 h, which indicated the 0.2 equiv of excess TPMA was not sufficient to promote the reaction to reach completion (Table 1, entry 11).

For most photo-ATRP or PET-RAFT reactions, continuous irradiation was required for the initiation and regeneration of active Cu(I) species. The major benefit of this system is the possibility of stopping or restarting the reaction at any time by simply turning the light off or on. The temporal control experiment (OEOMA₅₀₀, DP_T = 80, Cu = 1000 ppm) showed no additional conversion after turning off the light source, while the reaction progressed smoothly after the light was switched on (Figure 3A). In the first 30 min the conversion was limited because of the induction period; afterward the reaction rate increased rapidly whenever the irradiation was turned on. After 2 h of total irradiation time the polymerization reached 85% conversion ($M_{n,Th} = 34000$) and produced polymers with $M_n = 32\,400$ and $M_w/M_n = 1.17$. Subsequently, the reaction mixture was directly supplemented with 0.5 mL of a second monomer OEOMA₃₀₀ in order to confirm the retention of the chain-end functionality. The chain extension continued under blue light for another 2.5 h and finally formed a block copolymer with $M_n = 59\,500$ and $M_w/M_n = 1.28$ without any tailing or shoulder from the first peak's region (Figure 3B). The combination of temporal control and chain extension experiments confirmed the “living” polymerization mechanism as well as the excellent efficiency of the deoxygenation during the process.

Control experiments were carried out in order to optimize the polymerization conditions. Glucose could act as reducing agent to initiate ARGET ATRP also in organic solvents.²⁰ Thus, a control experiment was launched using the exact same conditions of Table 1, entry 1, but with the addition of an aluminum foil cover to avoid any external irradiation (Table S1, entry 1). This reaction was stirred for 15 h, and 71% conversion was acquired. The polymer was characterized as $M_n = 38\,900$ (vs $M_{n,Th} = 73\,200$) and $M_w/M_n = 1.32$. This indicated that glucose can be used to reduce Cu(II) to Cu(I), but its efficiency is limited. In the next control experiment, GOx and sodium pyruvate were removed from the reaction, but blue light was provided (Table S1, entry 2). In the absence of GOx and sodium pyruvate, no conversion was observed in 48 h, which indicated that glucose and blue light could not trigger ATRP in the presence of oxygen. These control experiments have confirmed the necessity of GOx as well as blue light for the fast and well-controlled ATRP process. To test the feasibility of the blue-light photo-ATRP system to synthesize protein–polymer hybrids, an ATRP initiator-modified bovine serum albumin (BSA-[iBBR]₃₁) to graft from its surface was used as a macroinitiator. The ATRP initiator linker, which was previously reported, contained a cleavable ester moiety and anchoring *N*-hydroxysuccinimide (NHS) species to assist covalent coupling with accessible lysine residues on the surface of BSA.^{11a} The polymerization was conducted under photo-ATRP by blue light using Glu + GOx + SP for deoxygenation (Figure 4). In 2 h, the polymerization reached 93% conversion, and kinetic samples at every 30 min were taken and then analyzed through nuclear magnetic resonance

(NMR), GPC, and DLS. The results showed a linear semilogarithmic kinetic plot versus time (Figure 4A). The molecular weight of the polymer was analyzed by cleaving the polymer chains from the enzymatic structure under mild basic conditions (5% NaOH) as measured by DMF GPC (Figure 4C). The evolution of M_n versus conversion confirmed the formation of well-defined polymers from protein with predetermined molecular weight ($M_n = 89\,300$) and low dispersity at 1.17 (Figure 4B). Diameters of the protein—polymer hybrids were analyzed by DLS. From initiator-modified BSA (10 ± 2 nm), the diameter of the protein—polymer conjugate was gradually increased to 50 ± 4 nm (Figure 4D).

In addition to protein—polymer conjugates, DNA—polymer conjugates were also prepared using blue light. A 23-mer DNA macroinitiator was synthesized with a preattached α -bromoisobutyrate (iBB) group on the 5'-end as previously reported.²¹ The polymerization reaction was performed in small disposable culture tubes of borosilicate glass with a reaction volume of 150 μ L. Polymerization was performed under highly dilute conditions with a DNA macroinitiator concentration of 20 μ M. The reaction was stopped after different time intervals and was analyzed using aqueous GPC, yielding well-defined DNA—polymer hybrids around $M_n = 75\,000$, $M_w/M_n = 1.18$ after 30 min, and $M_n = 110\,000$, $M_w/M_n = 1.23$ after 45 min (Table S2, Figure 4E).

In conclusion, the first example of photo-ATRP using blue LED irradiation in aqueous media is reported. Well-defined polymers were synthesized after several hours at room temperature. The reactions were simplified and shortened by the application of a Glu + GOx + SP deoxygenating system. As compared to more energetic light sources, blue light is friendlier to biological systems and allows enzymes to survive and preserve their structures and functions. Moreover, successful synthesis of protein— and DNA—polymer conjugates was reported accomplished with excellent control of molecular weights and dispersities of polymer segments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors thank Dr. James Spanswick and Emilee Tkacik for help with editing the manuscript. The financial support from NIH (R01DE020843) is gratefully acknowledged.

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Figure 1. Circular dichroism (CD) measurements for (A) BSA and (B) GOx under irradiation from various light sources for 2 h at room temperature.

Figure 2.
 (A) Semilogarithmic kinetic plots and (B) evolution of M_n and M_w/M_n with conversion of two different deoxygenation methods.

Figure 3.

(A) Semilogarithmic kinetic plots of photo-ATRP of OEOMA₅₀₀ by switching light source on/off and (B) GPC traces of one-pot chain extension of the final polymer from (A) with a second block of OEOMA₃₀₀.

Figure 4.

Grafting from ATRP initiator-modified protein (BSA) and DNA with photo-ATRP under 450 nm irradiation. (A) Semilogarithmic kinetic plot, (B) evolution of M_n and M_w/M_n with conversion, (C) GPC traces of cleaved polymers, and (D) evolution of diameters of protein—polymer conjugate particles over the reaction progress, measured by DLS. (E) GPC traces of DNA macroinitiator (DNA-iBBR) and DNA-pOEOMA₅₀₀ conjugates after 30 and 45 min of reaction.

Table 1.

Results of Photo-ATRP of OEOMA₅₀₀ under 450 nm Irradiation with Various Conditions^a

entry	$M_0/I_0/Cu_0/TPMA_0$	solvent	initiator	DP _T	Cu (ppm)	conv. % ^b	$M_{n,Th} \times 10^{-3c}$	$M_{n,GPC} \times 10^{-3}$	M_w/M_n
1	200/1/0.2/1	H ₂ O + 30 mM NaBr	PEG _{2k} BPA	200	1000	86	88.2	78.4	1.22
2	200/1/0.2/1	H ₂ O + 30 mM NaBr	PEG _{2k} iBBR	200	1000	91	93.4	71.6	1.20
3	200/1/0.2/1	1X PBS	PEG _{2k} iBBR	200	1000	86	88.3	89.6	1.16
4	200/1/0.2/1	1X PBS	PEG _{2k} BPA	200	1000	93	94.7	91.4	1.13
5	100/1/0.1/0.5	1X PBS	PEG _{2k} BPA	100	1000	89	46.7	47.2	1.19
6	600/1/0.6/3	1X PBS	PEG _{2k} BPA	600	1000	93	281.2	225.3	1.23
7	400/1/0.4/2	1X PBS	PEG _{2k} BPA	400	1000	91	182.4	170.9	1.23
8	400/1/0.2/1	1X PBS	PEG _{2k} BPA	400	500	86	174.2	161.2	1.19
9	400/1/0.04/0.2	1X PBS	PEG _{2k} BPA	400	100	50	100.5	104.3	1.31
10	400/1/0.02/0.1	1X PBS	PEG _{2k} BPA	400	50	8	10.1	75.5	1.54
11	200/1/0.2/1.2	1X PBS	PEG _{2k} BPA	200	1000	55	56.9	55.7	1.21

^a[M] = 20 vol %, total volume = 5 mL, reaction time = 2 h, T = r.t., irradiated at 450 nm at 10.0 mW/cm².^bConversion determined by ¹H NMR using <2% DMF as internal standard.^c $M_{n,Th}$ calculated based on equation ($M_{n,Th} = M_{initiator} + [OEOMA500]_0/[Initiator]_0 \times \text{conversion} \times M_{monomer}$). $M_{n,GPC}$ and M_w/M_n are determined by GPC in DMF, based on linear PMMA as the calibration standard.