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Visible-Light-Induced Cysteine-Specific Bioconjugation: Biocompatible Thiol-Ene Click Chemistry **

Hangyeol Choi,[a,b] Myojeong Kim,[a,b] Jeabong Jang[,b] and Sungwoo Hong*[,b,a]*

***Abstract**: Bioconjugation methods using visible-light photocatalysis have emerged as powerful synthetic tools for the selective modification of biomolecules under mild reaction conditions. However, the number of photochemical transformations that allow successful protein bioconjugation is still limited because of the need for stringent reaction conditions. Herein, we report that a newly developed water-compatible fluorescent photosensitizer **Q**$_{PEG}$ can be used for visible-light-induced cysteine-specific bioconjugation for the installation of **Q**$_{PEG}$ by exploiting its intrinsic photosensitizing ability to activate the S–H bond of cysteine. In addition, the slightly modified **Q**$_{CAT}$ enables the effective photocatalytic cysteine-specific conjugation of biologically relevant groups. The superior reactivity and cysteine selectivity of this methodology was further corroborated by traceless bioconjugation with a series of complex peptides and proteins under biocompatible conditions.*

Introduction

The chemical modification of biomolecules has become crucial for the advancement of chemical biology, molecular biology, and drug development. In particular, a hybrid of proteins and synthetic molecules (i.e., drugs and probes) provides a powerful tool for protein research, pharmaceutical chemistry, and biotechnology. For example, the fluorescent labeling of proteins enables the analysis of protein function, structure, dynamics, and trafficking pathways as well as the development of biological assays for drug screening.[1] In addition, the successful development of antibody-drug conjugates has fueled the development of reliable methods amenable to the bioorthogonal and chemoselective protein modification.[2] For the development of more diverse conjugation techniques, continued efforts have been made to modify natural amino acids by precisely controlling the position and number of molecules on proteins.[3] Recently, visible-light-induced bioconjugation has an attractive feature in terms of environmental sustainability and mild reaction conditions, as illustrated in Figure 1a.[4-6] This strategy proves effective in generating reactive radical species that can participate in a unique bond-forming process, leading to novel strategies in biomolecule functionalization. Despite the elegant merits, photoredox-catalyzed biorthogonal reactions are still complicated by some challenging problems. One of the major obstacles is the use of stoichiometric amounts of base or oxidant, which is often incompatible with the delicate nature of proteins and the tolerability of amino acid residues. Another limitation is the poor solubility of catalysts and reagents in aqueous solutions, and therefore many of the photochemical methods developed for biomolecule-material conjugation only occur in organic media, which limit the biochemical applications. In this regard, the development of the water-compatible and additive-free protocol is highly desirable for the visible-light-mediated.

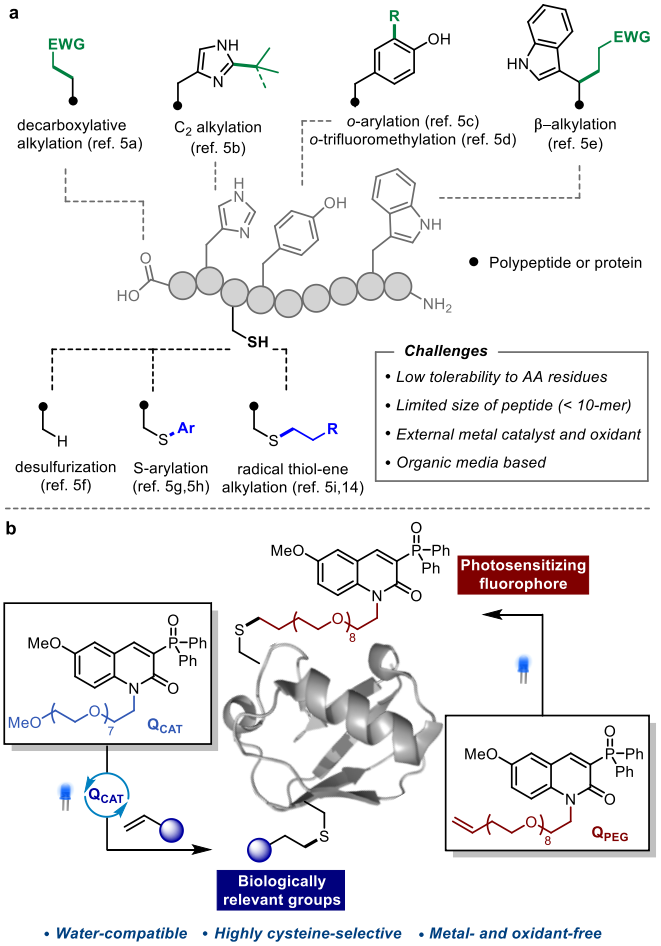

Figure 1. Visible-light mediated site-specific and chemoselective bioconjugations. a) Current strategies for visible-light-induced site-specific conjugation. b) A new strategy for visible-light-induced cysteine-specific bioconjugation of photosensitizing fluorophore **Q**$_{PEG}$ and **Q**$_{CAT}$-catalyzed cysteine-specific conjugation. EWG = electron withdrawing group.

Cysteine is a crucial residue for the chemical modification in selectively stitching two molecules together, and it has been exploited in various fields such as bioconjugate chemistry, medicinal chemistry, and polymer chemistry.[7] In traditional cysteine bioconjugation, the most employed strategy is the thio-Michael addition to maleimide acceptors by utilizing excellent nucleophilicity of the thiol group. Although this method has been widely investigated, there are significant concerns of a lack of chemoselectivity in the presence of nucleophilic amino acids and the inherent instability of the resulting thiosuccinimide linkage.[8] For the last few years, several remarkable studies provided newly developed reagents and efficient cysteine bioconjugation methods for the improved stability of protein

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conjugates.[9] Among them, radical thiol-ene reaction for C–S bond formation is one of the most promising cysteine modification methods, because radical insertion does not compete with other nucleophilic residues and leads to more stable adducts.[7b,10] However, highly oxidizing conditions often hamper the stability of oxidation-labile residues and cause detrimental effects on proteins a result of concurring side reactions.[10b,11] Moreover, direct chemical modification of natural amino acids in native proteins requires biocompatible reaction conditions (i.e., mild temperature, physiological pH, aqueous buffered solutions, and high dilution) and functional group tolerance to preserve protein structures, which makes challenging the development of photomediated bioconjugation means. In this context, the chemoselective generation of thiyl radicals under mild and water-compatible conditions represents a significant challenge to accomplish efficient cysteine bioconjugation via photomediated radical thiol-ene reaction.

Recently, our group has developed an organic photocatalyst **Q$_1$**, based on a quinolinone chromophoric unit and successfully achieved diverse visible-light-induced functionalization of pyridines under mild and metal-free conditions.[12] This photocatalyst **Q$_1$** exhibits blue fluorescence (λ_{abs} = 380 nm; λ_{em} = 448 nm) with a quantum yield of 0.28 in 1:1 (v/v) dimethylformamide/phosphate buffer (20 mM, pH 7.5) solvent mixture (see Figure 2b). Drawing inspiration from these observations, we hypothesized that incorporating a suitable pendant alkene moiety onto the photosensitizing fluorophore **Q$_1$** backbone would enable the direct assembly of fluorophore-labeled biomolecules by functioning bioconjugation process at the cysteine residues. The **Q$_1$**-derived water-compatible photosensitizers, **Q$_{PEG}$**, and **Q$_{CAT}$** are capable of performing direct hydrogen-atom transfer (HAT) upon excitation with blue LEDs and selectively generating radicals on cysteine residues for biologically relevant cysteine-conjugation, as shown in Figure 1b. Herein, we report a novel visible-light-induced cysteine bioconjugation for the installation of a fluorophore, **Q$_{PEG}$** by exploiting its intrinsic photosensitizing ability in aqueous media under metal-, oxidant- and external photocatalyst-free conditions. In addition, the use of water-compatible photocatalyst **Q$_{CAT}$** allowed cysteine conjugations of biologically relevant groups enabled by photocatalytic radical thiol-ene reaction under biologically ambient conditions. The convenience of this protocol was further illustrated by conjugating an important class of cysteine-containing peptides and proteins. Remarkably, the obtained **Q$_{PEG}$**-conjugates provide dual functionality for producing photoluminescence and singlet molecular oxygen ($^1O_2$) under visible-light irradiation, highlighting potential applications in image-guided photodynamic therapy.

Results and Discussion

We initially examined the photocatalyzed thiol-ene reaction between *N*-acetyl-*L*-cysteine **1** and alkene **2** using various organic photocatalysts (**Q$_1$**, Eosin Y, and Mes-Acr$^+$) for exploring the proposed concept, as shown in Figure 2a. When **Q$_1$** was irradiated with blue LEDs, we were pleased to observe the formation of the desired conjugate **3** in 86% yield. Remarkably, an external oxidant was not required in this protocol, thereby potentially avoiding side reactions of peptides bearing oxidation-sensitive residues. Because the presence of various nucleophilic functional groups found in biomolecules can significantly limit the scope and efficiency, we next investigated the chemoselectivity of the current method by adding amino acid additives such as Tyr, His, Ser, Asp, and Lys bearing multiple reactive functional groups. Importantly, the cysteine-specific conjugation process using **Q$_1$** was proceeded in high conversion yields regardless of adding other amino acids, and no detectable modifications occurred on other amino acids (see the Supporting Information for details, Figure S2). The orthogonal reactivity, along with compatibility with various functional groups, highlighted possible applications of **Q$_1$** towards cysteine-specific bioconjugation and modification of peptides and proteins.

Based on the encouraging results, we reasoned that **Q$_1$**-photochemistry could be applied to the fluorescent labeling of biomolecules, in which the **Q$_1$**-derivative serves as both an organic photosensitizer and a fluorophore. Indeed, **Q$_{ENE}$**, a **Q$_1$**-analog possessing a terminal alkene linker, promoted the desired reaction with 1 in dilute concentration of acetonitrile (10 mM), leading to the formation of the desired **Q$_{ENE}$**-cysteine conjugate in 77% yield after an hour (see SI for details, Table S2, entry 2). Whereas this preliminary result is encouraging, **Q$_{ENE}$** was visibly insoluble when increasing the ratio of phosphate buffer in the reaction solvent, and therefore the desired conjugation was completely unreactive (Table S2, entries 4 and 5). In this context, from the outset of our studies, the identification of suitable photosensitizers that enable aqueous bioconjugation processes was a priority (Table S2, entries 7-10). Through modulation of water-soluble groups directly attached to **Q$_1$**, **Q$_{PEG}$** (Figure 2b), a derivative bearing octa-ethylene glycol (PEG-8), was found to be visibly soluble in phosphate buffer with 10% v/v acetonitrile, and was capable of producing conjugated adduct **4** in 71% yield under the blue LED irradiation at room temperature for 3 h (Table S2, entry 11). Importantly, water-compatible fluorophore, **Q$_{PEG}$** exhibited blue fluorescence (λ_{abs} = 378 nm; λ_{em} = 450 nm) with a high quantum yield (Φ_F = 0.4) in aqueous media (Figure 2b and Table S4).

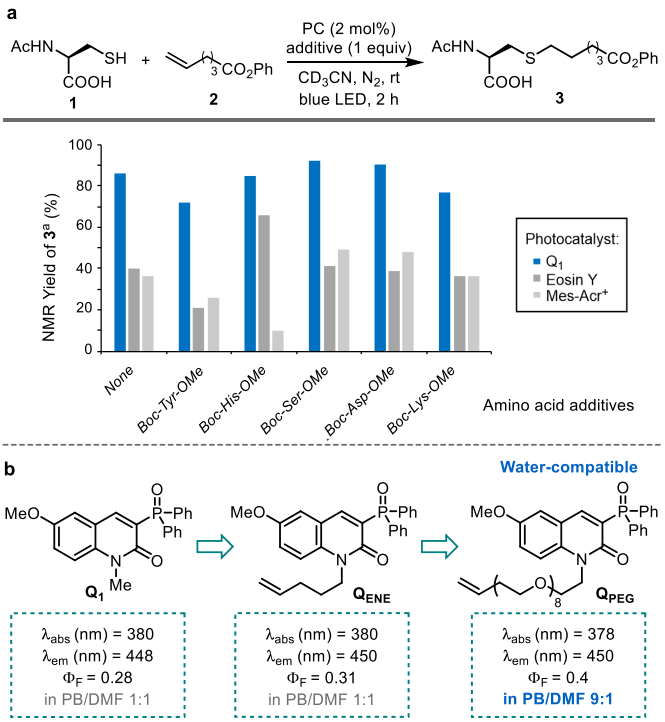
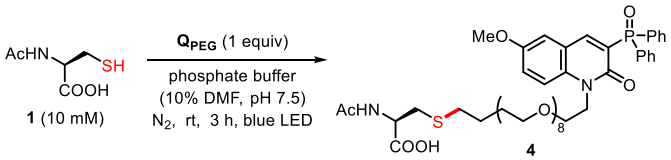


Figure 2. Visible-light induced thiol-ene reactions with cysteine. a) Survey of tolerance for the cysteine-specific thiol-ene reaction in the presence of competing nucleophilic amino acid additives. Reaction conditions: **1** (100 mM), **2** (100 mM), photocatalyst (2 mol%), and amino acid additive (100 mM) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2 for 2 h. Yields were determined by ^{1}H NMR spectroscopy. b) Development of **Q$_{PEG}$**. Mes-Acr$^+$ = 9-Mesityl-10-methylacridinium Perchlorate. Boc = *tert*-butoxycarbonyl.

As a result of modification of fluorophore, **Q$_{PEG}$** was selected as the photosensitizing fluorophore in our study, and we further investigated the optimization of the reaction conditions as outlined in Table 1. The irradiation with blue-light for 3 h promoted the formation of the

desired product **4** with yields of 87% under phosphate buffer with 10% dimethylformamide (entry 1). Among the co-solvents screened, acetonitrile and dimethyl sulfoxide were slightly inferior to dimethylformamide (entries 2 and 3). The use of pyridinium salt as an oxidant, a key component in **Q$_1$**-mediated reactions,[12] was not required for this transformation (entry 4). The use of 2 equiv of **1** dramatically improved the formation of the desired product with a yield of 95% (entry 5), but we kept using **1** as a limiting substrate for applying this technique in bioconjugation. We next investigated the effect of pH on the reaction because solubility and conjugation of substrates can be pH-dependent. To our delight, the desired adduct **4** was generated in comparable yields regardless of the pH (pH 3.5 NaOAc buffer, pH 5.5 HEPES buffer, or pH 8.8 Tris buffer) (entries 6-8). The necessity of light for product formation was confirmed by a control experiment (entry 9). The addition of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) completely inhibited the reaction, suggesting that a radical pathway is operational in this conjugation reaction (entry 10).

Table 1: Optimization of the reaction conditions[a]



Entry	Deviations from the standard conditions	Yield (%)[b]
1	none	87
2	MeCN instead of DMF	71
3	DMSO instead of DMF	70
4	pyridinium salt (1 equiv)	65
5	2 equiv of **1**	>95
6	NaOAc buffer (pH = 3.5) instead of PB buffer	81
7	HEPES buffer (pH = 5.5) instead of PB buffer	78
8	Tris buffer (pH = 8.8) instead of PB buffer	76
9	no light	NR
10	addition of TEMPO (2 equiv)	NR

[a] Reaction conditions: **1** (10 mM), and **Q$_{PEG}$** (10 mM) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2. [b] Yields were determined by ^{1}H NMR spectroscopy using 1,1,2,2,-tetrabromoethane as a standard. DMSO = Dimethyl sulfoxide. DMF = dimethylformamide. pyridinium salt = *N*-ethoxy-2-methylpyridinium tetrafluoroborate. PB = phosphate buffer. HEPES = (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

To elucidate the reaction pathway, we further conducted several mechanistic studies. Stern-Volmer fluorescence quenching experiments (see Figure S18) did not show any meaningful quenching of **Q$_{PEG}$** along with increasing concentrations of **1**, indicating that classical single electron transfer (SET) and energy transfer pathways may not participate in the reaction mechanism. The evaluation of the excited state reduction potential of **Q$_{PEG}$** from the cyclic voltammetry (see Figure S19) and the absorption spectra (see Figure S17) analysis, could exclude the possibility involving the SET process with a thiol group of cysteine. Therefore, we speculated that the triplet excited state of **Q$_{PEG}$** might serve as a direct HAT reagent to activate a labile S–H bond of cysteine, forming the corresponding thiyl radical in the reaction media. Thus, the thiyl radical generated through a direct HAT process[13] of **Q$_{PEG}$** under blue LED irradiation, acts as an electrophile towards the alkene of **Q$_{PEG}$**, leading to the formation of the C–S bond and the generation of the alkyl radical adduct **A**. In the chain pathway supported by the calculated reaction quantum yield (Φ_R = 4.13), the alkyl radical **A** abstracts a hydrogen atom from another thiol group to deliver the conjugation product **4** and release the thiyl radical that can start a new chain pathway. Alternatively, the alkyl radical **A** abstracts hydrogen from the intermediate **Q$_{PEG}$**–H through a reverse hydrogen atom transfer (RHAT) event to furnish the product and regenerate the ground-state **Q$_{PEG}$** to complete the catalytic cycle, as shown in Figure 3.

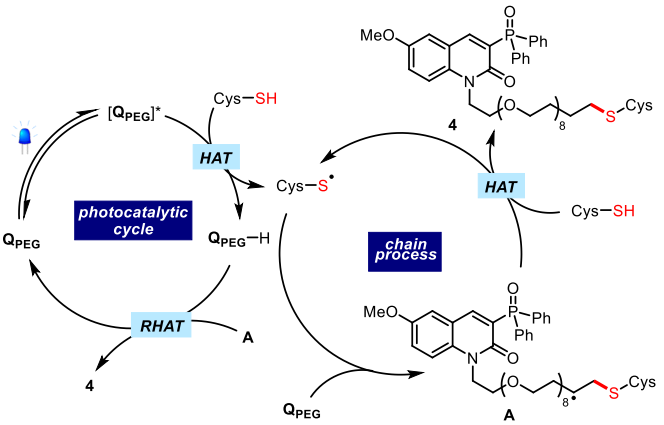


Figure 3. Proposed mechanism for the cysteine-specific conjugation of **Q$_{PEG}$**. HAT = hydrogen-atom transfer. RHAT = reverse hydrogen-atom transfer.

With the optimized conditions in hand, we next examined the applicability of this photocatalytic methodology for an endogenous tripeptide, glutathione (GSH) to investigate the tolerability of two carboxylic acids and free-amine functionalities that are present at the *C*- and *N*-termini of proteins, as shown in Table 2. We were pleased to observe the conjugation reaction of **Q$_{PEG}$** with GSH proceeded smoothly in 73% yield. Encouraged by this result, the scope of the method and functional group tolerance were next investigated with peptide substrates bearing naturally occurring amino acids. Remarkably, cysteine conjugation reactions were efficiently achieved with **Q$_{PEG}$** under pH 7.5 at room temperature. Both hydrophobic (Gly, Ala, Leu, Ile, Val, Met, and Pro; **6a-6g**) and hydrophilic (Ser, Thr, Asp, Glu, Asn, Gln, Lys, and Arg; **6h-6o**) side chains of natural amino acids were well tolerated in these conjugation reactions. Moreover, our results demonstrated that the tetrapeptides containing challenging amino acids such as Ser, Lys, Trp, His, and Tyr that often exhibit the deleterious effects on the bioconjugation outcome, were able to efficiently participate in the desired conjugation with **Q$_{PEG}$** at pH 7.5 (**6h**, **6n**, **6p**, **6q**, and **6r**). It had been previously reported that lowering the pH to 3.5 was required for improving the photoredox conjugation of peptides containing Lys, His, and Tyr residues to prevent oxidation of these sensitive residues.[5a] Any conjugates arising from the additions of other nucleophilic residues were not detected despite the presence of multiple nucleophiles in the peptide sequence, as confirmed by LCMS analysis, demonstrating the excellent selectivity for the cysteine conjugation. Further evaluation of the generality of the reactions with tetrapeptides containing cysteine residues at *C*- or *N*-termini, revealed that **Q$_{PEG}$** could be efficiently conjugated with cysteine regardless of its position in the sequence. We next applied the optimized conditions to more complex peptides such as fragments of endogenous proteins derived from insulin (**6u**, octapeptide) and Src kinase (**6v**, dodecapeptide). Both peptides were successfully conjugated with **Q$_{PEG}$** under our optimized reaction conditions in yields of 70% and 62%, respectively. For **6s** and **6v**, the conjugation reactions were performed in an acidic NaOAc buffer (pH 3.5) due to the insolubility of the substrates in a neutral phosphate buffer (pH 7.5).

In nature, most cysteine residues in endogenous proteins create disulfide bonds that contribute to the formation of a stable three-dimensional structural fold of the proteins.[14] As expected, **Q$_{PEG}$** was not able to react with a disulfide bond under optimal conditions, exhibiting that the **Q$_{PEG}$**-mediated C–S bond formation is highly

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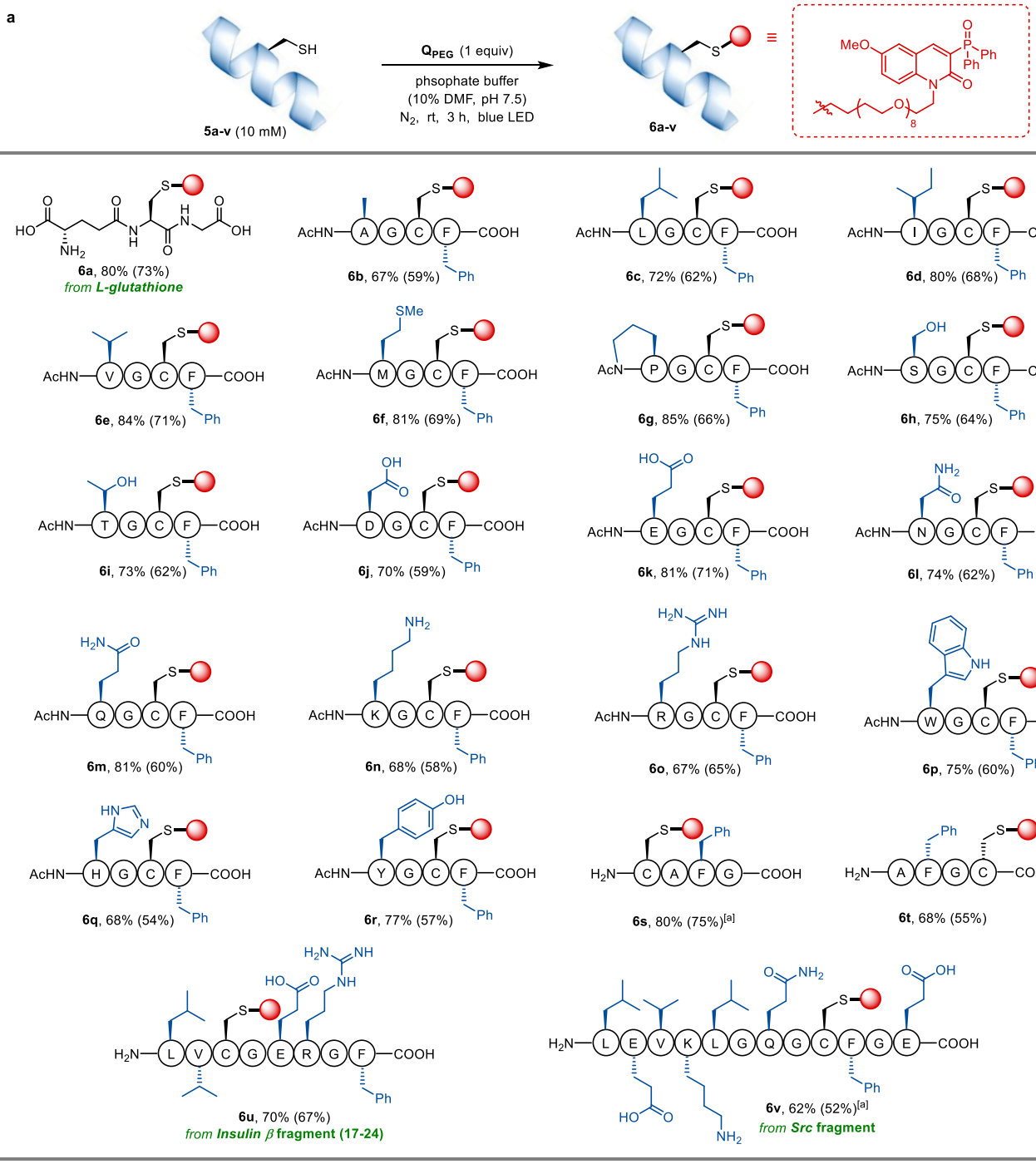
Table 2: Scope and functional group tolerance in polypeptides[a]



[a] Conjugation of free-cysteine-containing polypeptides. Reaction conditions: **5a-v** (10 mM) and Q_{PEG} (10 mM) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2 for 3 h. [a] NaOAc buffer (pH 3.5) was used instead of phosphate buffer. [b] Conjugation of disulfide-containing molecules. Reaction conditions: **7** (6.7 mM), Q_{PEG} (13.4 mM), and TCEP (6.7 mM) in phosphate buffer (10% DMF, pH 7.5) were stirred at rt under N_2 for 2 h and with irradiation by a blue LED (440 nm, 8.5 W) for 3 h. [b] **7c** (2 mM), Q_{PEG} (4 mM), and TCEP (2 mM) in NaOAc buffer (10% DMF, pH 3.5). Yields were determined by ^{1}H NMR spectroscopy. The isolated yields by HPLC are provided in parentheses. TCEP = tris(2-carboxylethyl)phosphine.

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selective to the free-thiol of cysteine over the disulfide (Table 2b).To further demonstrate the applicability of the current method, additional experiments were designed to determine if the process was compatible with the protein disulfide reducing agent tris(2-carboxyethyl)phosphine (TCEP)[15] and could enable conjugation of disulfide-containing molecules. Indeed, **Q$_{PEG}$** efficiently underwent reaction with *N*-acetyl-*L*-cystine **7a**, and *L*-glutathione oxidized **7b** under the presence of TCEP, leading to the formation of the corresponding C–S bond in good yields (**4**, 81% and **6a**, 79%). Moreover, neurohyphophysial nonapeptide hormone oxytocin **7c** could efficiently be conjugated with two **Q$_{PEG}$** fluorophores (**8**, 60%), even in highly diluted concentration (2 mM). Taken together, this protocol could offer a general strategy for the bioconjugation of a photosensitizing fluorophore.

Combining properties of a fluorophore and a singlet oxygen ($^1O_2$) photosensitizer can provide a unique opportunity for image-guided therapeutic applications.[16] We reasoned that the obtained photosensitizing fluorophore **Q$_{PEG}$**-conjugates could enable the photosensitized production of singlet oxygen upon light irradiation. In this regard, we evaluated the $^1O_2$ generation ability of **6a** in aqueous media, as shown in Figure 4. The photogeneration of singlet oxygen by **6a** was confirmed by the production of oxidation product TEMPO, which was detected by electron paramagnetic resonance (EPR) spectroscopy (unique triplet signal with a_N = 16.9 G and g = 2.00056, Figure 4b and Figure S24).[17] Next, to quantify singlet oxygen production, singlet oxygen sensor green (SOSG) was used as an indicator, which displayed excellent singlet oxygen generation ability of **6a** under visible-light irradiation (Figure 4c and Figure S25).[18]

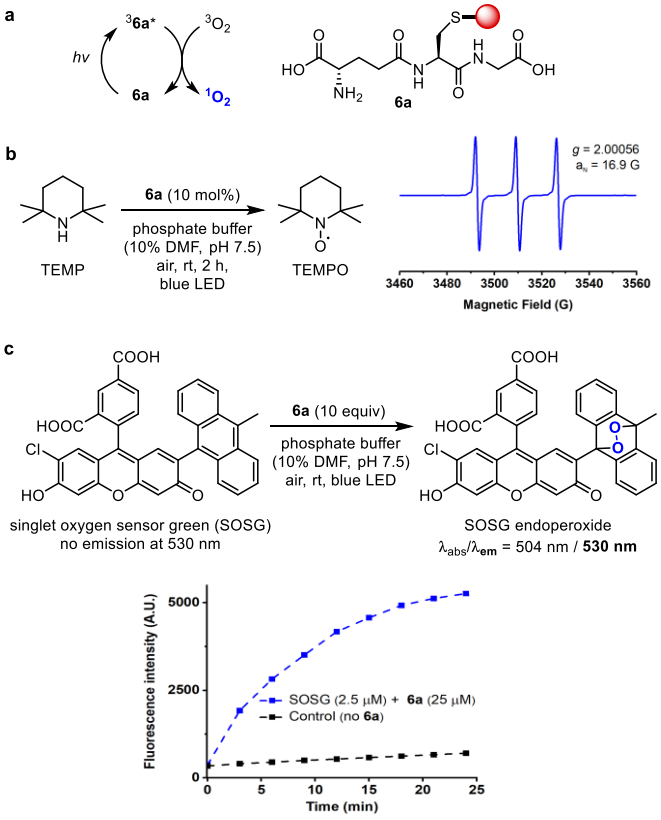
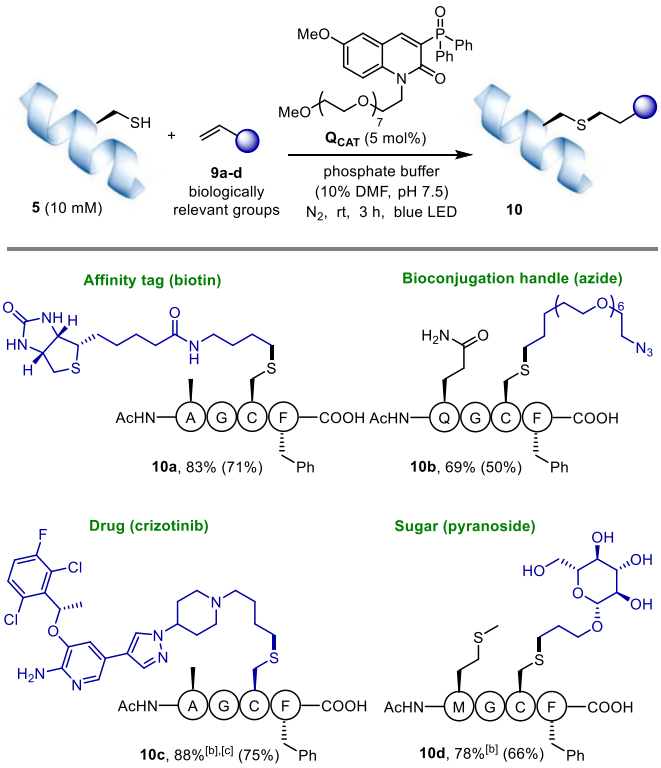


Figure 4. Singlet oxygen generation by Q$_{PEG}$-conjugated GSH **6a**. a) Photoinduced $^1O_2$ generation by **6a**. b) Photogenerated TEMPO from TEMP and EPR spectra of the TEMPO radical. c) Fluorescence detection of singlet oxygen generation using SOSG. Reaction conditions: SOSG (2.5 μM) and **6a** (25 μM) in aerated phosphate buffer (pH 7.5, 10% v/v dimethylformamide) with irradiation by a blue LED (415 nm) under air for 30 min. TEMP = 2,2,6,6-tetramethylpiperidine.

Next, we speculated that the **Q$_{PEG}$** could be used as a water-compatible photocatalyst for the cysteine conjugation of biologically relevant groups in aqueous media. For this purpose, we slightly modified **Q$_{PEG}$** by removing its olefin group and assessed the photocatalytic ability by evaluating conjugation of peptides with a variety of important biologically relevant molecules such as affinity tag (biotin), bioorthogonal handle (azide), crizotinib (ALK inhibitor), and sugar. As illustrated in Table 3, we were pleased to observe that **Q$_{CAT}$** was shown to be competent in photocatalytic cysteine conjugations, affording the corresponding adducts under the standard reaction conditions. The current photocatalytic strategy, therefore, presents a valuable and versatile platform for selective cysteine conjugation of biologically relevant groups as well as fluorophore **Q$_{PEG}$** under biologically ambient conditions.

Table 3: Photocatalytic conjugation of diverse biological substrates[a]



[a] Reaction conditions: **5** (10 mM), **10** (11 mM), and **Q$_{CAT}$** (5 mol%) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2 for 3 h. [b] **Q$_{CAT}$** (10 mol%) was used. [c] NaOAc buffer (pH 3.5) was used instead of phosphate buffer.

To test the applicability of our method to tertiary structural proteins, the compatibility of **Q$_{PEG}$** and **Q$_{CAT}$** in protein bioconjugation was evaluated using ubiquitin K63C (Ub K63C)[19] and bovine serum albumin (BSA), as illustrated in Figure 5. Native ubiquitin (Ub) is a small size globular protein of 76 residues (8.6 kDa) found in most tissues. Serum albumin is a globular protein found in blood plasma, and fluorophore-labeled albumin conjugates have been widely used for biological imaging.[20] BSA contains only one exposed surface cysteine (Cys34) and 17 conserved disulfides. We aimed to link the photosensitizing fluorophore **Q$_{PEG}$** directly to cysteine residues in these proteins using the developed visible-light-induced protocol. For this purpose, Ub K63C was mixed with **Q$_{PEG}$** in 10% DMSO in phosphate buffer and incubated under blue light irradiation for 6 hr. Pleasingly, an analysis of the mixture using LCMS showed a mass corresponding to the conjugate of Ub K63C–**Q$_{PEG}$** (Figure 5a and Figure S8). The labeled Ub K63C–**Q$_{PEG}$** was clearly observed after trypsin digestion by peptide mapping ESI-MS spectrometry to identify the site of conjugation. With a similar protocol utilizing **Q$_{PEG}$**

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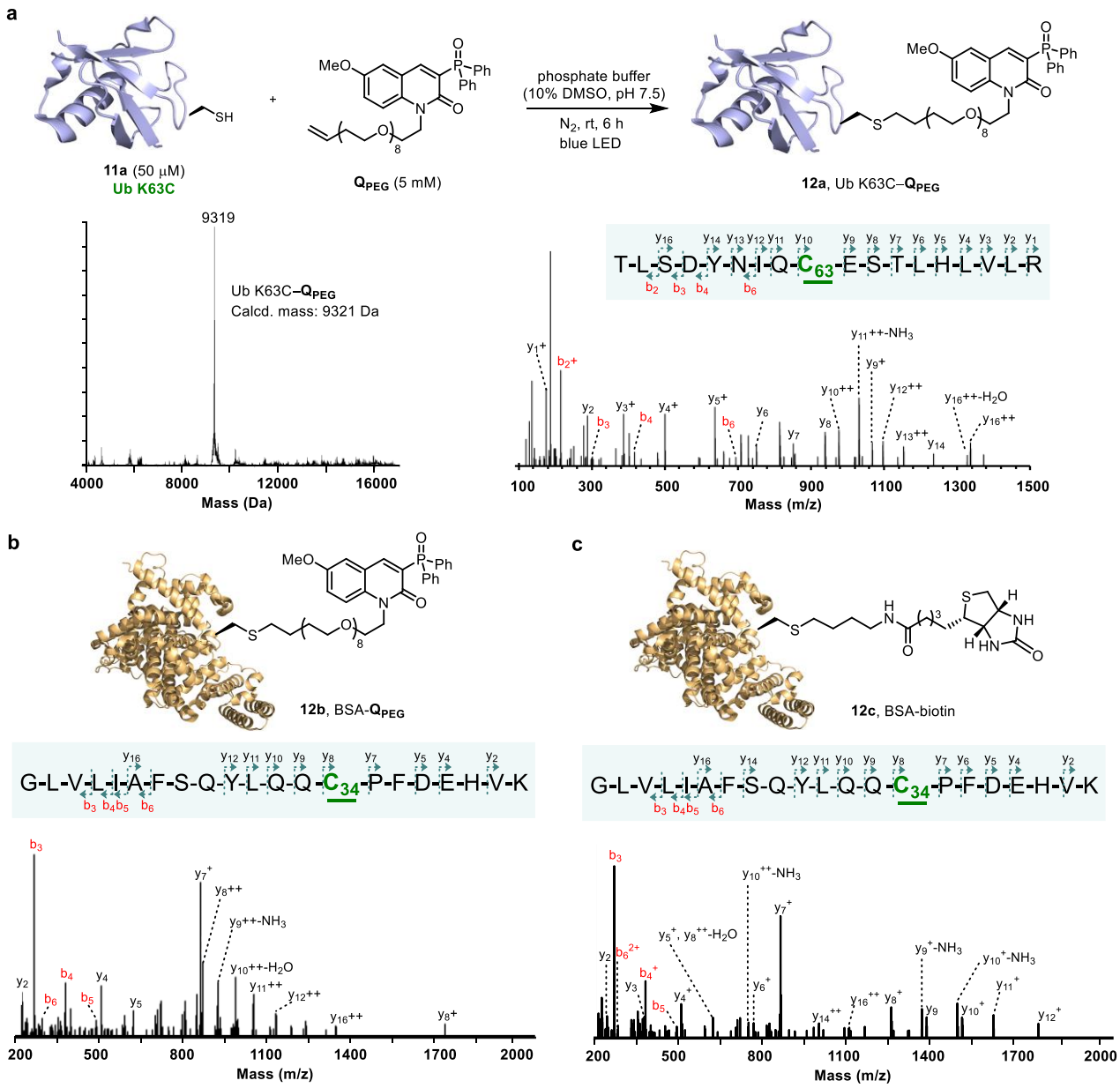

Figure 5. Visible-light-induced site-specific bioconjugation on proteins. a) Functionalization of Ub K63C with **Q**$_{PEG}$. Ub K63C (50 μM) and **Q**$_{PEG}$ (5 mM) in phosphate buffer (10% DMSO, pH 7.5) with irradiation by a blue LED (440 nm, 8.5 W) for 6 h. Deconvoluted LCMS mass spectrum for the Ub K63C-**Q**$_{PEG}$ conjugate (Product: 9321 m/z; $[M+781]^+$), MS/MS spectrum of the tryptic peptide TLSDYNIQCESTLHLVLR, modification at the cysteine residue of Ub K63C-**Q**$_{PEG}$. b) Functionalization of BSA with **Q**$_{PEG}$. BSA (100 μM) and **Q**$_{PEG}$ (2 mM) in phosphate buffer (10% DMF, pH 7.5) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2 for 6 h. MS/MS spectrum of the tryptic peptide GLVLIAFSQYLQQCPFDEHVK, modification at the cysteine residue of BSA-**Q**$_{PEG}$. c) Functionalization of BSA with **Q**$_{CAT}$ and **11a**. BSA (100 μM), **Q**$_{CAT}$ (2 mM) and **11a** (2 mM) in phosphate buffer (10% DMF, pH 7.5) with irradiation by a blue LED (440 nm, 8.5 W) at rt under N_2 for 6 h. MS/MS spectrum of the tryptic peptide GLVLIAFSQYLQQCPFDEHVK, modification at the cysteine residue of BSA-biotin.

and **Q**$_{CAT}$, Cys34 of BSA was readily labeled with **Q**$_{PEG}$ and biotin, respectively (Figure 5b and Figure 5c). These results revealed that Ub K63C and BSA were successfully tagged with a single **Q**$_{PEG}$ or biotin in the presence of 50 to 100 μM protein, a concentration commonly used for the labeling of proteins.

Conclusion

In summary, we have discovered a fluorescent photosensitizer **Q**$_{PEG}$ and photocatalyst **Q**$_{CAT}$ capable of mediating visible-light-induced cysteine-specific conjugation under biocompatible reaction conditions. By exploiting the intrinsic photosensitizing capacities of **Q**$_{PEG}$ and **Q**$_{CAT}$, a fluorophore **Q**$_{PEG}$ and biologically relevant groups could be installed chemo- and regioselectively into a series of complex peptides and proteins. In the process, the exclusion of external oxidant and base is critical to suppress undesired reaction of the peptide and protein substrates. This metal-free and operationally simple conjugation proceeds with high efficiency, chemoselectivity, and functional group tolerance, providing great promise in many chemical disciplines for the preparation of fluorescent photosensitizer- or biomolecule-bound peptide and protein conjugates.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: photosensitizer • cysteine • bioconjugation • photocatalysis • thiol-ene click chemistry

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Photochemistry

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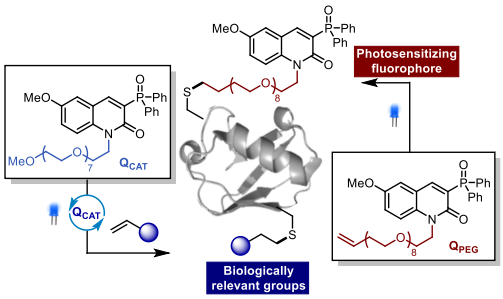
Visible-Light-Induced Cysteine-Specific Bioconjugation: Biocompatible Thiol-Ene Click Chemistry



Visible-light-induced cysteine-selective bioconjugation has been achieved using fluorescent photosensitizer $\mathbf{Q_{PEG}}$ and photocatalyst $\mathbf{Q_{CAT}}$. By exploiting the intrinsic photosensitizing capacities of $\mathbf{Q_{PEG}}$ and $\mathbf{Q_{CAT}}$, a fluorophore $\mathbf{Q_{PEG}}$ and biologically relevant groups could be installed chemo- and regioselectively into a series of complex peptides and proteins under biocompatible mild conditions.