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Site-selective tyrosine bioconjugation via photoredox catalysis for native-to-bioorthogonal protein transformation

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The growing prevalence of synthetically modified proteins in pharmaceuticals and materials has exposed the need for efficient strategies to enable chemical modifications with high site-selectivity. While genetic engineering can incorporate non-natural amino acids into recombinant proteins, regioselective chemical modification of wild-type proteins remains a challenge. Herein, we use photoredox catalysis to develop a site-selective tyrosine bioconjugation pathway that incorporates bioorthogonal formyl groups, which subsequently allows for the synthesis of structurally defined fluorescent conjugates from native proteins. A water-soluble photocatalyst, lumiflavin, has been shown to induce oxidative coupling between a previously unreported phenoxazine dialdehyde tag and a single tyrosine site, even in the presence of multiple tyrosyl side chains, through the formation of a covalent C–N bond. A variety of native proteins, including those with multiple tyrosines, can successfully undergo both tyrosine-specific and single-site-selective labelling. This technology directly introduces aldehyde moieties onto native proteins, enabling rapid product diversification using an array of well-established bioorthogonal functionalization protocols including the alkyne–azide click reaction.

Chemically modified proteins represent a growing class of biologics widely used in areas spanning cellular tracking, imaging, biomaterials, and drug therapeutics. Accordingly, chemical technologies or coupling strategies that enable efficient and highly site-selective modification of structurally complex proteins have recently found increased utility[1,2]. In particular, bioorthogonal synthesis (BOS) has emerged as a powerful approach to construct biohybrid conjugates, due in part to the capacity of bioorthogonal functional-group pairs (or click partners) to undergo chemoselective couplings while being inert to the innate structural elements of biology (for example, peptides, nucleic acids or sugars)[3]. Among the prerequisites for successful BOS applications, the site-specific incorporation of a click-type functional group into the designated bioarchitecture is the most critical. In terms of protein modification, most BOS groups are typically installed during protein biosynthesis through the implementation of unnatural amino acids via genetic engineering. An attractive complementary approach would involve the direct installation of bioorthogonal or click handles onto fully formed wild-type proteins in a positionally selective fashion (Fig. 1a). While this approach remains a challenging proposition, a number of recent studies have demonstrated its feasibility in a series of biological applications[4–7].

Although amino-acid-specific bioconjugation has evolved greatly over the past decade, developing a chemoselective and single-site-selective transformation remains a prominent and ongoing challenge. Alkylation methods to functionalize solvent-exposed residues such as lysine, while highly efficient, tend to lack regioselectivity[8]. By contrast, cysteine modification has become an attractive strategy for site-selective bioconjugation reactions owing to its lower abundance in proteins. However, this presents potential challenges in structural disruptions as Cys are generally engaged in important disulfide bonds[9]. As a result, recent protein conjugation efforts have moved beyond classical nucleophile–electrophile-based approaches to focus on highly specific click-paired coupling reactions, which target less-surface-abundant, redox-active residues. Elegant examples include functionalization at the N- (ref. [10]) and C- (refs. [11,12]) termini, as well as on tyrosine[13–17], tryptophan[18], and methionine[19,20] residues.

Over the last ten years, photoredox catalysis[21] has been shown to be a unique platform for the development of site-selective, functional-group-specific coupling protocols[22] that broadly operate via the production and trapping of open-shell intermediates. This general activation mode has been explored in several bioconjugation contexts, including peptide macrocyclization[23], and DNA-encoded library syntheses[24], both of which take advantage of the biocompatibility and mild conditions of photoredox catalysis (that is, aqueous media, low temperature and neutral pH). Recently, we speculated that photoredox catalysis could be employed to enable protein bioconjugation in a highly positionally selective manner, using a small-molecule coupling partner that would also incorporate a bioorthogonal or click functional group. If successful, this linchpin strategy would enable wild-type proteins to be BOS ready in one chemical step without the prerequisite for genetic engineering and unnatural amino acid incorporation (Fig. 1b). Moreover, the merger of site-selective native protein activation with known bioorthogonal click technologies would greatly streamline the synthesis of chemically modified proteins for a wide variety of applications.

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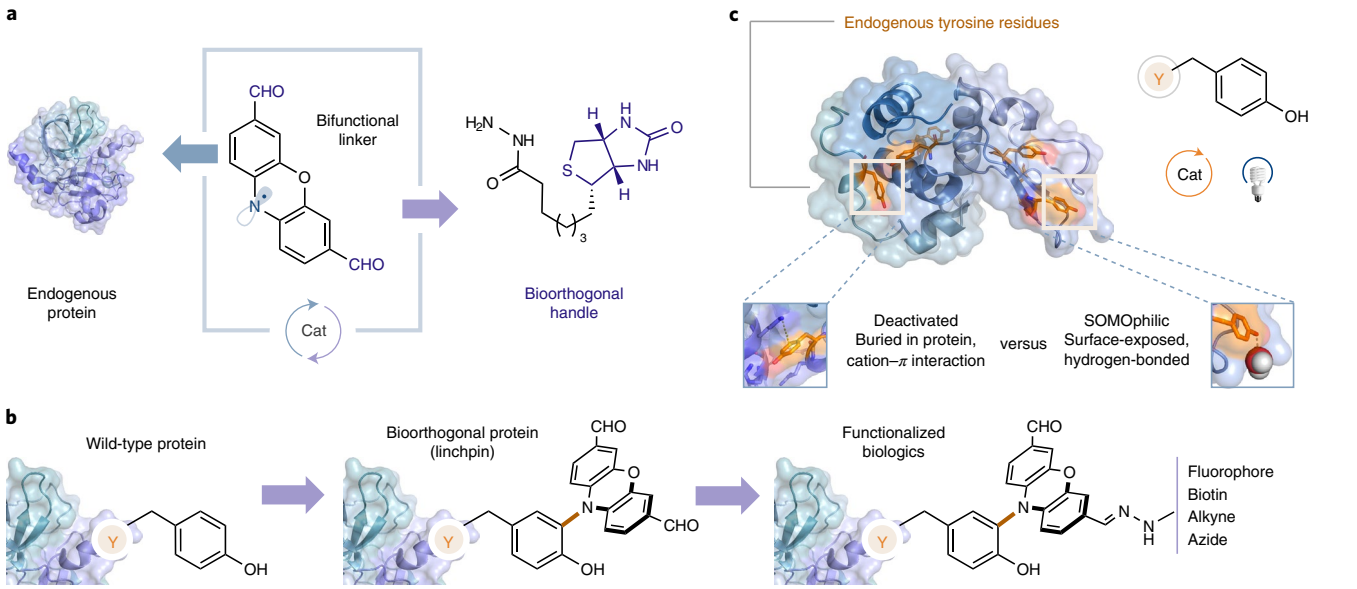


Fig. 1 | The photoredox-catalysed merger of native protein site-selective bioconjugation with bioorthogonal methodology. a, The merger of a site-selective photoredox bioconjugation method with bioorthogonal chemistry would rapidly generate structurally defined biohybrids directly from endogenous proteins. Cat, catalyst. **b**, This single-step chemical modification allows for the transformation of native proteins to incorporate bioorthogonal handles without genetic modification and incorporation of unnatural amino acids. **c**, Tyrosine residues are an attractive target to achieve single-site-selective modification via photoredox catalysis. Due to their amphiphilicity, tyrosines often exist in a variety of microenvironments, resulting in a range of reactivity. Those that are buried or engaged in cation–π interactions are deactivated, whereas surface-exposed or hydrogen-bond-donating tyrosines are SOMOphilic and readily reactive.

The specifics of our one-step 'native-to-bioorthogonal' protein modification strategy are outlined in Fig. 1. We hypothesized that the tyrosine residue would be a suitable candidate for developing a photoredox-catalysed site-selective bioconjugation protocol because, along with other methods, tyrosine (Tyr) has been known to undergo oxidative coupling under biochemical[25], electrochemical[15,16], and photochemical[17] conditions. Its SOMOphilicity (that is, its propensity to react with open-shell radical species) lends well to a single-electron-transfer (SET) platform. Regarding site-selectivity, we recognized that, in addition to its naturally low abundance in proteins (3.0% (ref. [26])), Tyr phenols are amphiphilic, thereby existing in a wide range of hydrophilic and hydrophobic microenvironments[27] (Fig. 1c). As such, we presumed that the various tyrosine residues found in any given protein structure would likely exhibit highly differentiated surface accessibility, π-electronics, and thereby SOMOphilicity. This would allow for both residue- and site-specific functionalization using only visible light, a biocompatible photocatalyst, and a suitable SET-activated, small-molecule coupling partner.

In contrast to the redox-gated reaction design in our previously reported C-terminus decarboxylative alkylation[11], which enabled selectivity by using C termini's relatively low oxidation potential, we hypothesized that photocatalyst-mediated oxidation of a suitable non-biogenic coupling partner might enable the use of an alternative SOMOphilicity-gated bioconjugation that is chemoselective for tyrosine. This potentially opens a challenging question regarding the regioselectivity between various Tyr residues on the same protein. We aim to use this complementary strategy to still achieve a site-selective bioconjugation without relying on the oxidation potential of the C-termini. Inspired by the recent literature on the use of heteroatom-centred radicals for highly selective aromatic C–H functionalization[28–30], we identified phenoxazine as a potential partner, given its capacity to form an N-centred radical upon electron and proton loss. More specifically, we hypothesized that an oxidative electron-transfer/proton-transfer (ET/PT)[31] event would generate an electrophilic N-centred radical that can selectively couple in a polarity-matched pathway with the electron-rich phenolic side chain of tyrosine, thereby forming a covalent C–N bond[32].

Encouraged by previous studies on phenoxazine–phenol couplings[33], we set out to design a multifunctional linchpin molecule that would both allow endogenous protein labelling and be amenable to BOS applications thereafter. We identified two crucial criteria in our linchpin molecule design: (1) reactivity that is not only tyrosine specific, but also selective between different Tyr residues on a native protein, and (2) incorporation of a versatile bioorthogonal handle for subsequent product derivatization. With these criteria in mind, we designed 10*H*-phenoxazine-3,7-dicarboxaldehyde (**1**, Table 1). In terms of design criteria, we hypothesized that incorporation of two electron-withdrawing formyl groups onto phenoxazine would, upon photochemical oxidation, lead to the formation of a more electrophilic *N*-centred radical in comparison to the parent unsubstituted arene, thereby encouraging polarity-matched[34,35], open-shell reactivity towards the generally electron-rich Tyr phenols (Supplementary Section 2 for details). Furthermore, aryl aldehydes have been shown in a number of studies to engage inefficient condensations with a wide range of fragments under biocompatible conditions[36]. As such, the incorporated aldehyde moieties would provide a versatile bioorthogonal handle upon phenoxazine coupling with endogenous proteins. Finally, the two aldehydes would extend phenoxazine π-conjugation, which, as we later verified, would render any modified protein adducts fluorescent. With this phenoxazine dialdehyde design, we set out to explore the SOMOphilicity-gated, tyrosine-selective pathway.

Results and discussion

After the successful synthesis of **1**, which can be rapidly obtained in three chemical steps from commercially available starting materials (Supplementary Section 9 for details), we began our investigations into site-selective tyrosine coupling using a small peptide pharmaceutical, bivalirudin (**3**, Table 1) as our model substrate. Given the

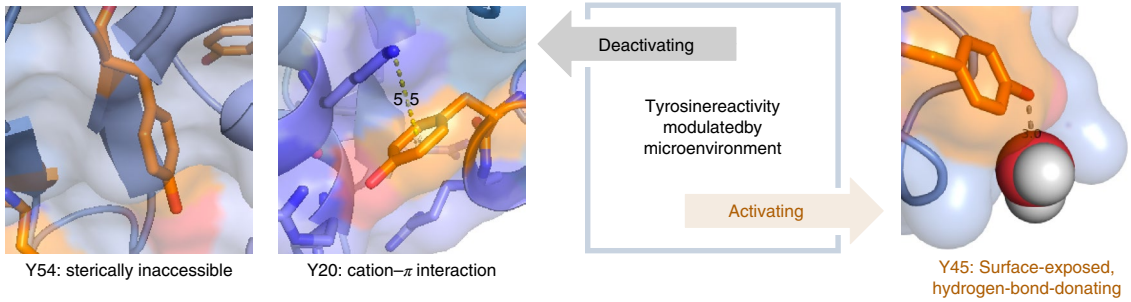


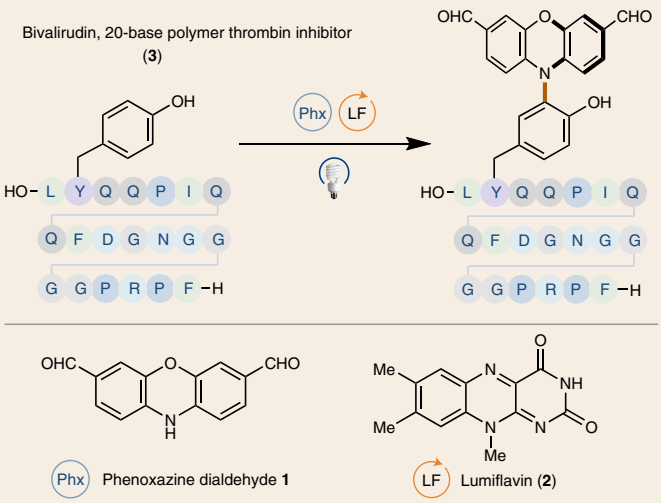
Fig. 2 | Tyrosine microenvironments. Representative tyrosines from human lysozyme (**6**) in their respective microenvironments. Y20 and Y54 are not reactive due to steric constraints or deactivating cation–π interactions, whereas Y45 is labelled with high efficiency because it is surface-exposed and hydrogen-bond-donating to surrounding aqueous media.

need for a highly oxidizing, water-soluble photocatalyst, we selected lumiflavin (LF, **2**, $E^{red}_{1/2}[^3LF/LF^{\bullet-}] = +1.68$ V, where $E^{red}_{1/2}$ is the reduction half-wave potential) for this oxidative reaction. Under optimized conditions (neutral pH and aqueous potassium phosphate buffer), bivalirudin (**3**) was found to undergo tyrosine-specific coupling with phenoxazine dialdehyde **1** at very high levels of efficiency (95% conversion, Table 1, entry 1). Notably, we observed no conversion to the coupled product in the absence of light or photocatalyst (entries 2 and 3), suggesting that the proposed SET pathway is operative.

With these results in hand, we next evaluated a variety of wild-type proteins with sizes ranging from 5.8 to 77.0 kDa, including those containing multiple tyrosine residues (Table 2). In each case, we report reaction efficiencies by taking advantage of the phenoxazine–phenol bioconjugate fluorescence (maximum absorbance $\lambda_{abs} = 430$ nm, maximum emission $\lambda_{em} = 525$ nm and quantum yield $\Phi = 4.9\%$). Conversion was reported based on the number of fluorescent tags per protein (Supplementary Section 3b). In addition, all protein substrates were recovered at >95% following reaction and purification. We determined the site-selectivity of our coupling reaction via trypsin digestion followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. With these characterization methods, we were delighted to observe high conversion with small proteins bearing a single tyrosine side chain (Table 2). For example, ZVar affibody **4**, a paratope antibody mimic, was effectively coupled in 83% conversion as a single product. While our methodology was readily found to translate from peptides to small proteins, we recognized that most proteins contain multiple tyrosine residues, presenting an additional, and most critical, challenge of selectivity. Again, as a key objective of our mechanistic hypothesis, we postulated better-than-statistical selectivity for a single residue position based on differential surface exposure and electron-density-derived SOMOphilicity.

At the outset, we were delighted to find that insulin (**5**, 5.8 kDa) underwent coupling events that were primarily selective at a single tyrosine site (Y14; B-chain in 6:1:0:0 regioselectivity) and with full conversion (4% double addition detected by fluorescence data and corroborated by intact mass analysis). This result was notable given that insulin contains a relatively high abundance of tyrosine residues (~8%, four in total). Gratifyingly, human lysozyme (**6**, 14.4 kDa) and ribonuclease A (**7**, 13.7 kDa), each bearing six tyrosine residues, were both efficiently labelled (62% and 53%, respectively) and, more importantly, with complete selectivity for a single tyrosine residue (Y45 and Y76, respectively). Moreover, cytochrome C (**8**, 12.4 kDa), a hemeprotein essential for electron transport in mitochondria, reacts with excellent selectivity at the Y74 residue under these photoredox conditions, albeit with trace labelling of two other Tyr residues (Y74/(Y48,Y97), 20:1). In the case of larger proteins, we found that α-lactalbumin (**9**, homohexamer, monomer

Table 1 | Optimized conditions and relevant control reactions

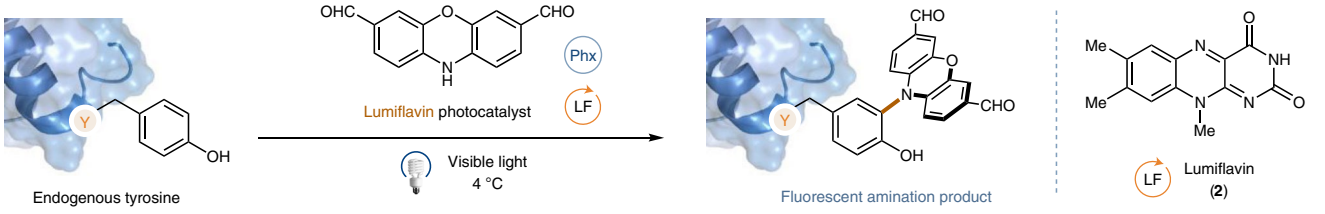


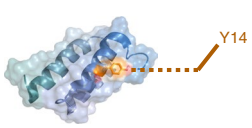
Entry	Deviation from standard conditions[a]	Conversion[b]
1	None	95%
2	Without light	0%
3	Without lumiflavin	0%

Initial optimizations indicating 95% conversion was achieved with our model substrate, bivalirudin (**3**, 10 nmol, 0.1 mM). [a]Standard conditions: phenoxazine dialdehyde **1** (10 mM), lumiflavin (**2**, 0.3 mM), KPi buffer (pH 7, 10 mM), 95:5 H_2O/dimethylformamide and 5 h irradiation with 34 W blue light-emitting diodes (440 nm) under N_2. [b]Conversion monitored by HPLC (254 nm).

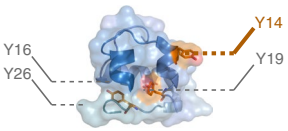
14.2 kDa) was also selectively functionalized to form predominantly one adduct despite the presence of three other tyrosines residues (Y18/Y103, 30:1, 84%). Hemeprotein myoglobin (**10**, 13.7 kDa) and chymotrypsinogen A (**11**, 25.7 kDa) were also amenable to these SET conditions and coupled efficiently to phenoxazine dialdehyde **1** (72% and 76% conversion, respectively). Myoglobin (**10**) was among the numerous systems that gave exclusive selectivity via the production of a single adduct at Y146. Chymotrypsinogen A (**11**) was also found to be selectively functionalized to the extent of Y146 (91% selectivity), with Y146 outcompeting three other tyrosine residues. Perhaps most notably, serotransferrin (**12**), the largest protein evaluated in our study (77.0 kDa) with 26 tyrosine residues, reacted with exquisite selectivity to furnish a single product (Y136, 56% conversion). The capacity to select one tyrosine among 26 Tyr residues speaks to the remarkable impact that protein microenvironments play on the inherent localized electronics of amino acid subunits embedded within a complex biological framework. Through the evaluation of our substrate scope, we observed that low reaction temperature (4 °C) and inert atmosphere are important for achieving

Table 2 | Scope of the photoredox-catalysed tyrosine amination as applied to native proteins

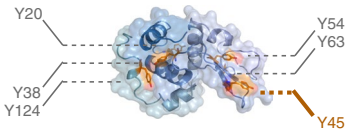




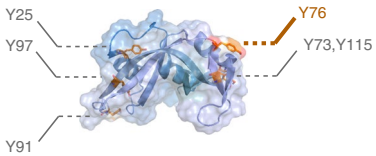
4, ZVar affibody (8.1 kDa)
1 tyrosine
76%[a]
Y14 single product



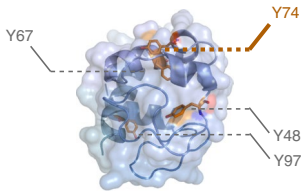
5, Human insulin (5.8 kDa)
4 tyrosines
100%[a]
Y14/Y19, 6:1



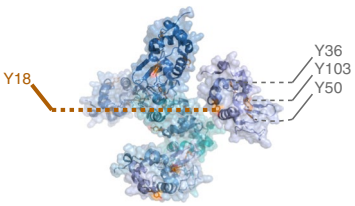
6, Human lysozyme (14.4 kDa)
6 tyrosines
65%[a]
Y45 single product



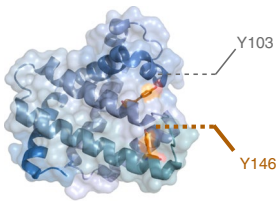
7, Ribonuclease A (13.7 kDa)
6 tyrosines
49%[a]
Y76 single product



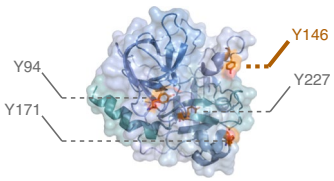
8, Cytochrome C (12.4 kDa)
4 tyrosines
64%
Y74/(Y48,Y97), 20:1



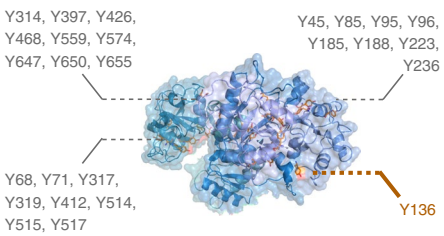
9,
4 tyrosines
89%[a]
Y18/Y103, 30:1



10, Myoglobin (13.7 kDa)
2 tyrosines
72%
Y146 single product



11, Chymotrypsinogen A (25.7 kDa)
4 tyrosines
76%[a]
Y146/Y171, 10:1



12, Serotransferrin (77.0 kDa)
26 tyrosines
55%
Y136 single product

A collection of endogenous proteins can be positionally modified at specific tyrosines under photoredox conditions in KPi buffer. Generalized reaction scheme and substrate scope[44]. Reaction conversion is obtained by comparing product emission against a fluorescence standard calibration curve (Supplementary Section 3b). Each substrate was repeated independently with similar results. [a]Conversions corroborated by intact mass spectrometry. Protein recovery >95% in all cases, according to BCA (bicinchoninic acid) assay. Site-selectivity on tyrosine is confirmed by trypsin digestion followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis.

good reactivity and minimizing substrate degradation such as oxidative damage. Finally, with all the protein systems we evaluated, the presence of other oxidizable side-chain moieties (such as Trp, Met, and carboxylates) do not interfere with the productive reaction pathway, further underscoring the residue-specific nature of these open-shell bond-forming processes.

The observation of single-site-selective reactivity of protein substrates with multiple tyrosines can be rationalized by a variety of stereoelectronic factors (Fig. 2). The amphiphilic phenol of tyrosine allows it to exist in a large variety of microenvironments, which we recognize would greatly impact reactivity. Those buried inside the protein tend to be sterically inaccessible for surface reactivity. Moreover, any cation–π interactions would deactivate tyrosine from formal oxidative amination by reducing the electron density within the π-system of the phenol. By contrast, the most reactive tyrosines are engaged in hydrogen bonding to either (1) negatively charged side chains or (2) water molecules in the surrounding aqueous media. Hydrogen-bond donation by the phenolic –OH group would increase electron density in the aromatic ring, thus increasing the SOMOphilicity of tyrosine towards oxidative cross-coupling with the electrophilic *N*-centred radical on phenoxazine dialdehyde **1**.

On the basis of literature reports and our own transient absorption spectroscopy experiments, we propose that the flavin photocatalyst **2** ($E^{red}_{1/2}[^3LF/LF^{\bullet-}] = +1.68$ V) is promoted to its singlet excited state through excitation with blue light, followed by intersystem crossing to form the triplet **13** (quantum yield of intersystem crossing, $\Phi_{ISC} = 0.68$ in water at pH 7, Fig. 3a)[36,37]. The triplet-excited flavin photocatalyst **13** could undergo electron and proton transfer with **1**, resulting in a lumiflavin semiquinone **14**, HFl$^{\bullet}$, and the

electron-deficient N-centred radical **15** after proton transfer. We presume that this electrophilic phenoxazinyl radical can couple with the electron-rich phenol side chain of tyrosine-containing protein **16**. After a final ET/PT between the radical semiquinone **14** and tyrosyl radical **17**, the conjugated tyrosine–phenoxazine adduct **18** is furnished. The resulting reduced lumiflavin, H_2Fl, **19** can be regenerated as the active lumiflavin photocatalyst **2** by way of a terminal oxidant such as molecular O_2, thereby closing the catalytic cycle.

When we considered the reaction mechanism, we recognized that the excited state of lumiflavin **13** (^{3}Fl) is capable of oxidizing both tyrosine and **1** (anodic peak potential, E_{pa}[Tyr/Tyr$^{\bullet+}$] = +1.01 V and E_{pa}[**1**/**1**$^{\bullet+}$] = +0.53 V, versus saturated calomel electrode in potassium phosphate (KPi) buffer, pH 7). This presented two possible reaction mechanisms: one that proceeds via the phenoxazinyl radical **15** and one via a tyrosyl radical[38]. We sought to delineate the two pathways using transient absorption spectroscopy, studying the differential decay of lumiflavin excited state absorbance in the presence of either reaction component (Fig. 3b). We observed that the excited state absorption of flavin **13** at 433 nm is quenched by **1**, and not by the tyrosine within insulin (**5**). This experimental result supports a radical pathway through the formation of an N-centred phenoxazinyl radical **15**, in agreement with our selectivity hypothesis. In addition, selective quenching of ^{3}Fl **13** by phenoxazine **1** is not observed at equimolar concentrations of **13** and **1** (Supplementary Section 7 for details).

Having achieved the site-selective bioconjugation on endogenous tyrosines with a variety of native proteins, we next sought to demonstrate the bioorthogonal utility of these adducts via application of the aforementioned aldehyde-click handle. The collection of research developed and inspired by Bertozzi and coworkers[39] has popularized the use of hydrazides and alkoxyamines for aldehyde condensations to form hydrazines and oximes under biocompatible conditions. On this basis, we were able to successfully incorporate a diverse array of small-molecule linkers (all commercially available) into our functionalized protein adducts. As one specific example, the single-site-functionalized human lysozyme bioconjugate from **6** was successfully modified with a variety of useful biofunctionalities, including a fluorescent cyanine dye, biotin, and azide/alkyne click handles (Fig. 4a). Employing a simple anthranilic acid catalyst[40] at neutral pH, we observed the full conversion of bioorthogonal-ready lysozyme to each corresponding derivative. The bioorthogonal reactivity of our phenoxazine formyl adducts was easily exploited, in that these subsequent aldehyde-click reactions could be readily performed with the crude photoredox reaction mixtures without purification. As such, the native-to-bioorthogonal concept could be extended further to allow a 'native-to-fully modified' transformation via a one-vessel approach.

Furthermore, we wish to highlight the biocompatibility of our method by emphasizing the mildness of this photoredox-catalysed method. All the proteins reported herein (**4**–**12**) were analysed via circular dichroism spectroscopy[41] before and after tyrosine modification. We were pleased to observe that all substrates retained their secondary structures after reaction (Supplementary Section 14 for details). We also subjected conjugated chymotrypsinogen A (**11**, 76%) to an enzyme activity test and found that, relative to the unmodified wild-type, 78% of the peptide cleavage function was retained (Fig. 4b). These characterizations establish that our tyrosine bioconjugates retain their structural and activity integrity, a crucial feature for downstream applications.

Finally, we note that the phenol–phenoxazine dialdehyde bioconjugates exhibit fluorescence, which, in addition to its use in measuring reaction efficiency, could also be used to track these biohybrids

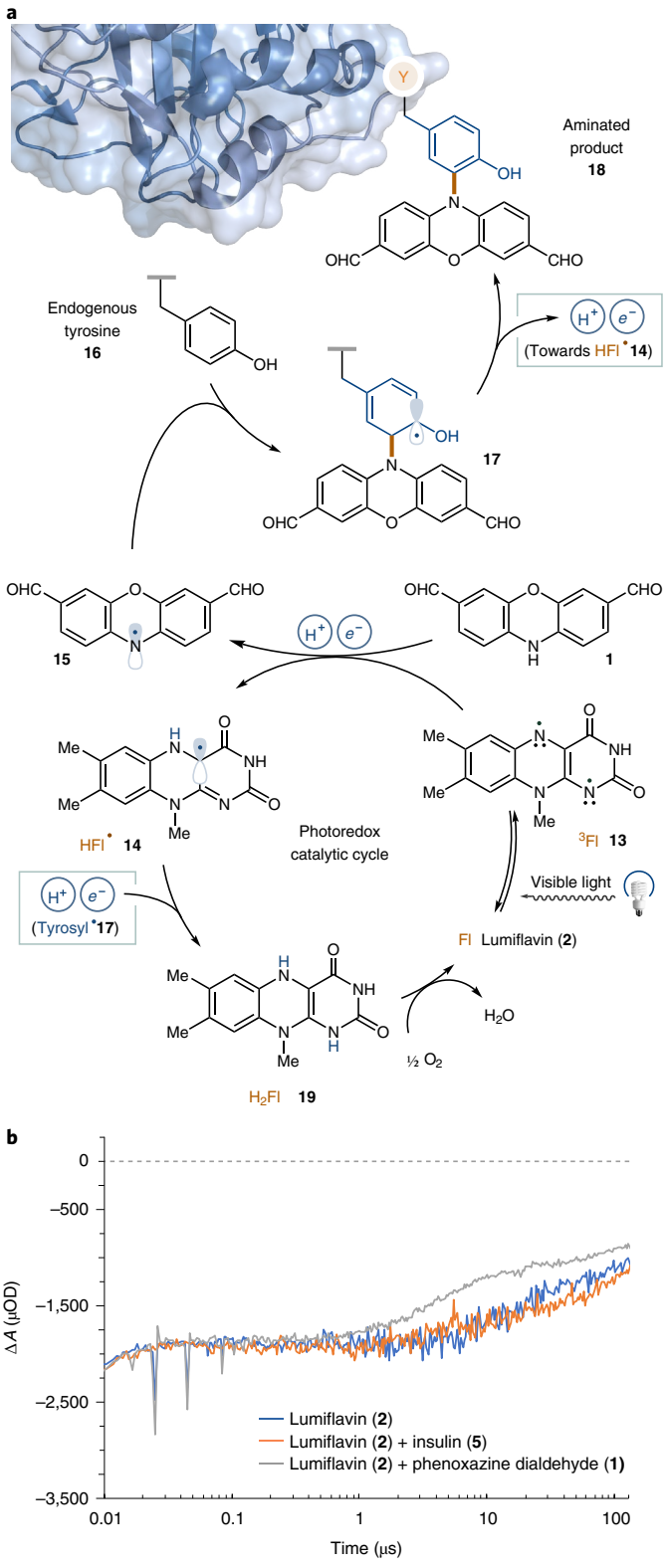


Fig. 3 | Proposed reaction mechanism for site-selective amination on tyrosine residues. a, Lumiflavin (**2**) is excited by blue light to its triplet excited state, which oxidizes **1** to produce N-centred radical **15**. This traps an accessible tyrosine **16** resulting in tyrosyl radical **17**, which donates a proton and electron to the semireduced HFl• **14**. This forms the bioorthogonal protein **18** and H_2Fl **19** that can be oxidized by O_2. **b**, Time-resolved transient absorption spectroscopy (pump/probe, 370 nm/433 nm) indicates that under reaction conditions (10 mM KPi buffer, pH 7, 95:5 H_2O/dimethylformamide), the lumiflavin excited state is quenched by **1** and not by insulin (**5**). This offers support for the formation of **15** versus protein oxidation. Δ*A*, change in absorbance; μOD, optical density units. See Supplementary Section 7 for additional experimental details.

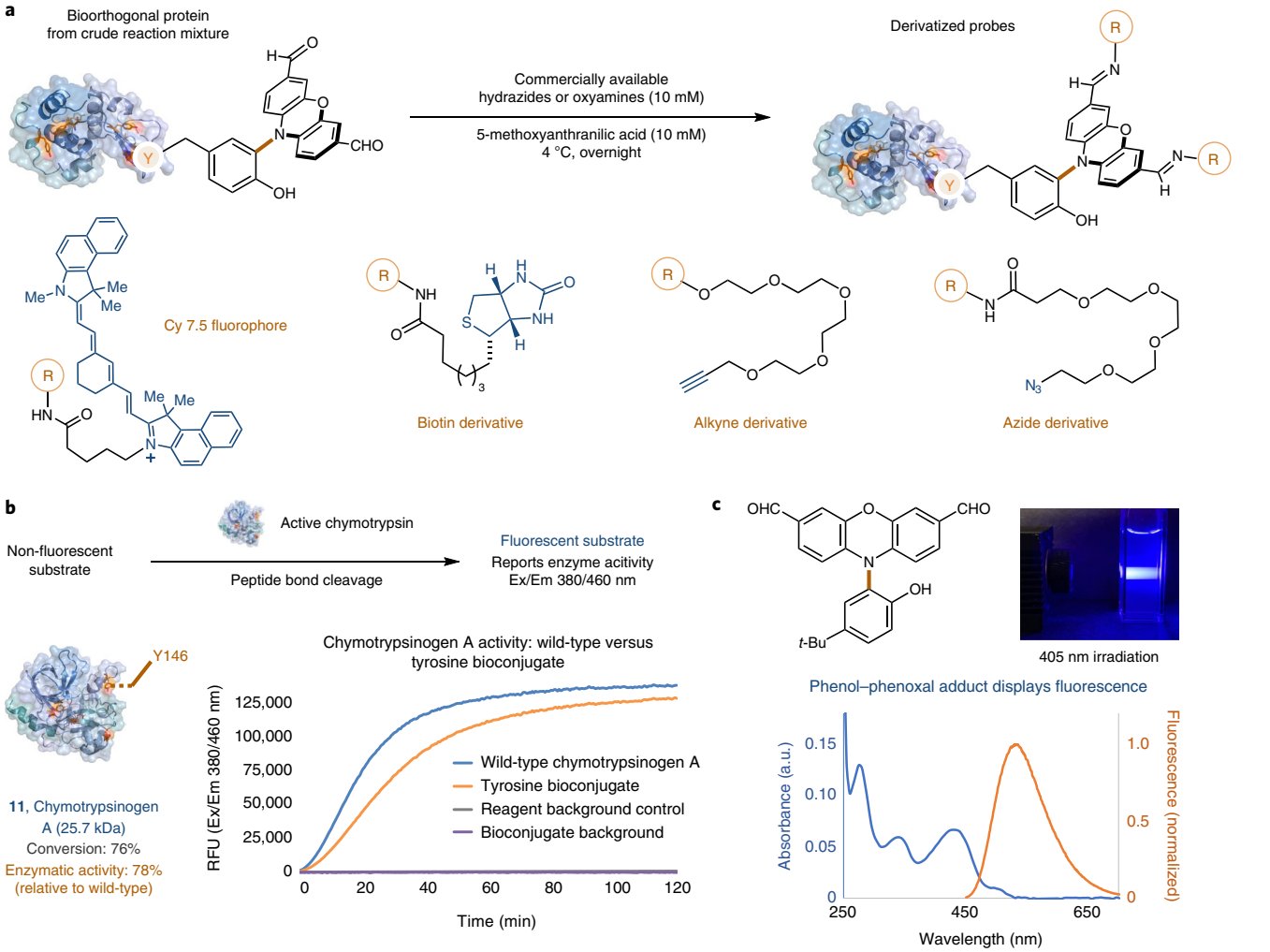


Fig. 4 | Diversification of photoredox tyrosine amination products via bioorthogonal aldehyde-click functionalization. a, Human lysozyme (**6**, 14.4 kDa), previously functionalized with our amination methodology, was subjected to operationally simple aldehyde-click reactions with various commercially available hydrazides or aminooxy biologics in a linchpin-selective fashion. Modifications were confirmed by fluorescence or mass spectrometry analysis. **b**, Chymotrypsinogen A (**11**, 25.7 kDa, 76%) retained 78% of the wild-type enzymatic activity towards peptide bond cleavage, as demonstrated by a commercial fluorescence assay (Supplementary Section 13 for complete experimental details) RFU, relative fluorescence units; Ex/Em, maximum excitation/emission wavelengths. **c**, A phenol–phenoxazine dialdehyde adduct displays fluorescence when irradiated with a 405 nm laser. It serves as a small-molecule standard for the characterization of fluorescent protein adducts (absorbance and fluorescence graph of adduct shown at bottom).

in a complex cellular environment. Initial characterizations of the adduct indicate that its green 525 nm emission should be suitable for fluorescent microscopy among other applications[42,43] (Fig. 4c). Complementary to genetically encoded fluorophores, this method generates fluorescent signals by chemically enhancing the wild-type protein's native tyrosine residue via the properties of the appended small-molecule system. Further applications of this phenoxazine dialdehyde photophysical behaviour are now being examined.

In conclusion, we have enabled the merger of endogenous and bioorthogonal labelling technologies via the use of photoredox catalysis. Using mild, low-temperature conditions in combination with aqueous buffer, we have site-selectively coupled a de novo tag (that is, phenoxazine dialdehyde) to the phenolic side chain of specific tyrosine residues on a wide range of native proteins. The single-site-chemoselective nature of this technology enables the synthesis of uniformly functionalized protein adducts. The capacity to select one tyrosine from among up to 26 tyrosine residues on a complex protein framework speaks to the remarkable impact that bio-microenvironments play on the inherent localized electronics and SOMOphilicity of amino acid subunits. Future work to extend visible light-mediated, site-selective bioconjugation methodology to encompass other native protein functionalities is now ongoing.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41557-021-00733-y.

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Data availability

The data supporting the findings of this study are available within the article and its Supplementary Information.

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Author contributions

B.X.L., D.K.K. and S.B. performed and analysed the bioconjugation experiments. R.Y.-C.H. performed and analysed the mass spectrometry experiments. D.G.O. performed and analysed the transient absorption spectroscopy experiments. D.K.K., B.X.L., S.B. and D.W.C.M. designed the experiments. D.K.K., B.X.L. and D.W.C.M. prepared this manuscript. G.D.S., J.X.Q. and W.R.E. provided helpful discussions.

Competing interests

The authors declare no competing interests.

Additional information

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