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Enzymatic Modification of N-Terminal Proline Residues Using Phenol Derivatives

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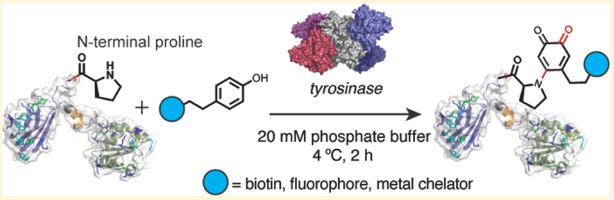
Supporting Information

ABSTRACT: A convenient enzymatic strategy is reported for the modification of proline residues in the N-terminal positions of proteins. Using a tyrosinase enzyme isolated from *Agaricus bisporus* (abTYR), phenols and catechols are oxidized to highly reactive *o*-quinone intermediates that then couple to N-terminal proline residues in high yield. Key advantages of this bioconjugation method include (1) the use of air-stable precursors that can be prepared on large scale if needed, (2) mild reaction conditions, including low temperatures, (3) the targeting of native functional groups that can be introduced readily on most proteins, and (4) the use of molecular oxygen as the sole oxidant. This coupling strategy was successfully demonstrated for the attachment of a variety of phenol-derivatized cargo molecules to a series of protein substrates, including self-assembled viral capsids, enzymes, and a chitin binding domain (CBD). The ability of the CBD to bind to the surfaces of yeast cells was found to be unperturbed by this modification reaction.



INTRODUCTION

The ability to construct site-specific protein bioconjugates has broad applicability in a range of disciplines. Fluorophores are commonly attached to proteins to study their localization in cells and biodistribution in living organisms,[1,2] and fluorescent labels can provide biophysical information about conformational changes.[2,3] Proteins are routinely immobilized on surfaces to probe mechanistic and binding features,[4,5] and increased efforts are exploring the utility of proteins as material components.[6] Most relevant to drug discovery is the synthesis of antibody−drug conjugates, where toxic payloads are covalently bound to malignant cell-type specific antibodies.[7,8] In all cases, the construction of a desired bioconjugate requires the specific folded conformation of the protein to be preserved to achieve proper function. Chemistries used to modify proteins must therefore proceed efficiently in aqueous conditions and under mild ranges of pH and temperature to minimize perturbations in protein structure.

A number of reactive strategies have been developed to meet these criteria. These reactions most commonly target lysine or cysteine residues, which are the two most nucleophilic side chains on the surfaces of proteins.[9] However, it can be difficult to control the modification numbers and locations using lysine-specific reactions, and the reliance of many proteins on cysteine side chains for proper function and folding can complicate the targeting of this residue. More recently, noncanonical amino acid (ncAA) mutagenesis[10−12] has emerged as a powerful technique to introduce new reactive handles in defined locations, allowing for site-selective modification when the appropriate bioorthogonal chemistry[13] is employed.

As a complementary approach, our lab[14−17] and others[18−21] have focused on the development of mild reactions that target the N-terminus as a chemically distinct site in a given protein sequence. Compared to the primary amines of lysine side chains, protein N-termini offer lower pK_a values and adjacent side chain groups that can participate in the reactions.[14,22] Furthermore, a variety of protein N-terminal amino acids can be introduced directly during protein expression. Studies have shown that if the amino acid following the initial methionine encoded by the start codon is small, such as alanine or proline, the methionine is removed completely in bacterial and mammalian expression systems.[23,24]

One class of reactions with particular promise for site-specific protein labeling involves the oxidative coupling of *o*-quinoid intermediates to nucleophilic functional groups. In these strategies, *o*-aminophenols or *o*-catechols are first oxidized through the use of protein-compatible oxidants, such as sodium periodate or $K_3Fe(CN)_6$.[15,25,26] The resulting *o*-iminoquinone or *o*-quionone species react rapidly with *p*-aminophenylalanine ncAAs,[25] N-terminal prolines,[15] and

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reduced cysteine residues,[15] as shown in Figure 1a (except in the thiol addition case, reported structures have been

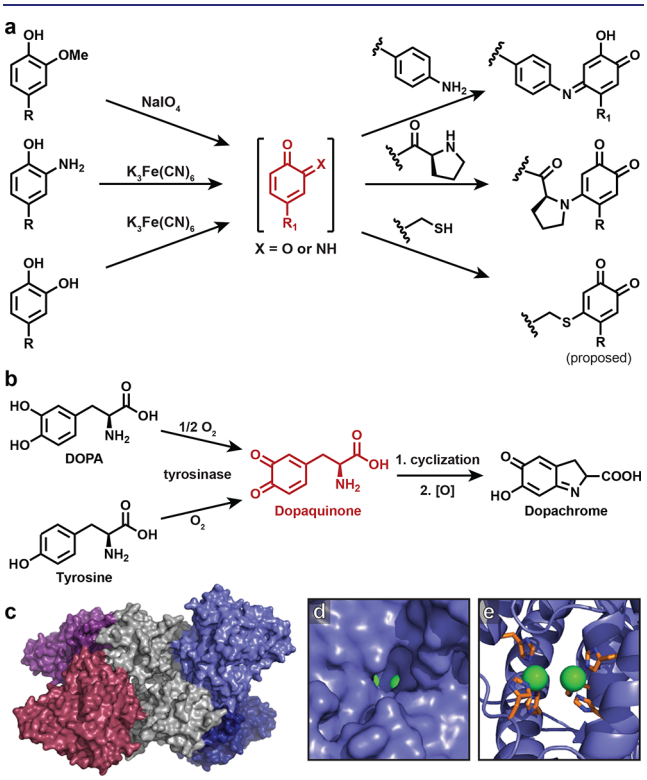

Figure 1. Chemoselective oxidative coupling reactions for bioconjugation and melanin biosynthesis. (a) Methoxyphenols, aminophenols, and catechols can be converted to *o*-quinoid intermediates in situ using periodate or the mild oxidant $K_3Fe(CN)_6$. These rapidly couple with anilines, N-terminal prolines, or thiols to form stable bioconjugation product. (b) A related pathway is catalyzed by tyrosinase as part of the melanin biosynthesis pathway. Both dopamine and tyrosine can serve as substrates for this reaction. (c) The tyrosine isolated from *Agaricus bisporus* is shown (PDB ID: 2Y9W). The inactive domains are depicted in gray. (d, e) The active sites consist of Type-III binuclear copper complexes ligated by histidine residues. These ions can be accessed via a deep pocket in the protein structure.

previously solved using NMR[15,25]). These reactions have been applied to the efficient modification of viral capsids with nucleic acids,[27] polymers,[28] and proteins;[29] inorganic nanoparticles with polymers and proteins;[30] and electrochemically active surfaces with oligonucleotides capable of cell capture.[31,32] Taken together, these studies highlight the utility of this class of reactions for the preparation of highly complex bioconjugates for many applications. These reactions also bear resemblance to catechol-based interactions involved in the adhesion of mussels to surfaces, which have found substantial use as adhesives that can function in aqueous conditions.[33,34]

Despite their success as bioconjugation partners, the *o*-aminophenol and *o*-catechol substrates used in these oxidative coupling reactions have key limitations. While both reagents are readily activated in the presence of the mild oxidant $K_3Fe(CN)_6$, they also oxidize in the presence of air over time. This limits their shelf life, while also adding challenges to the chemical preparation of these reagents. Indeed, when synthesizing aminophenol derivatives, our lab prefers to work with nitrophenols and then reduce them to *o*-aminophenols using sodium dithionite prior to reaction.[15,35] More recently, we reported *o*-methoxyphenols as alternative air-stable precursors to *o*-catechols.[26] In these cases, the *o*-methoxyphenol is oxidized to an *o*-quinone via a periodate-mediated reaction, in which the methoxy group is substituted with water. Further work on proteins has demonstrated that potentially deleterious side oxidations resulting from a large excess of periodate can be reduced if the oxidant is quenched with mannose following *o*-methoxyphenol activation and prior to protein reaction.[26] Despite these workarounds, however, this chemistry would still benefit from additional routes to the key *o*-quinone intermediates. Ideally, these strategies should feature substrates that are commercially available, allow reagent storage, and minimize the need for large excesses of oxidants that must be quenched and removed following the bioconjugation reaction.

Previous work has demonstrated that enzymes are powerful tools for oxidizing phenol-containing compounds, playing myriad roles in biology, including neurotransmitter synthesis,[36,37] toxin removal,[38] and the production of melanin.[39,40] In melanosomes, tyrosinase catalyzes the 4-electron oxidation of tyrosine to dopaquinone, which undergoes an intramolecular 1,4-conjugate addition to produce dopachrome, Figure 1b.[39,40] This molecule is then further polymerized to produce various melanin compounds across a variety of organisms. Tyrosinase can also catalyze the two-electron oxidation of dopamine to the same dopaquinone intermediate, demonstrating the ability of the enzyme to make the same *o*-quinone intermediates from both catechol and phenolic substrates.[39,41]

Herein we apply this biochemical concept to develop an enzymatic oxidative coupling strategy. Using a commercially available tyrosinase enzyme, the *o*-quinone intermediates required for oxidative coupling reactions can be accessed using simple catechol and phenol precursors. In addition to simplifying the substrate synthesis, this activation method uses only atmospheric oxygen as the oxidant and produces water as a reaction byproduct. Starting from commercially available NHS esters coupled to tyramine, this strategy is demonstrated through the attachment of a variety of phenolic derivatives to N-terminal proline residues in protein sequences. Finally, we extend this technique toward the construction of an N-terminally labeled chitin-binding domain (CBD) for use as a potential targeting agent for yeast and pathogenic fungal organisms.

RESULTS AND DISCUSSION

Screening the Enzymatic Oxidative Coupling with Small Molecule Substrates. Tyrosinase is conveniently available from commercial sources following isolation from the common button mushroom *Agaricus bisporus* (abTYR).[40,42] This variant is a ~120 kDa MW tetrameric protein containing multiple active subunits and inactive subunits. The enzyme has type III dicopper binding sites located in deep and spacious active sites, Figure 1c.[41,42] It is these large cavities that allow abTYR to act on a variety of phenols beyond tyrosine. Previous studies have shown abTYR is capable of binding and oxidizing acetaminophen, BPA, and even 17-β-estradiol,[43−45] indicating that the enzyme is promiscuous for a variety of phenol reagents with varying amounts of added complexity. This capacity for phenol oxidation has recently been exploited in the context of bioconjugation through the conversion of engineered, solvent-accessible tyrosine residues to *o*-quinones to allow hetero-

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Diels–Alder reactions with cyclooctyne derivatives.[46] This chemistry was demonstrated for the attachment of cargo molecules to C-terminal tyrosine residues extending from properly engineered antibodies. A tyrosinase-based approach has also been described to convert tyrosine residues in peptides and proteins to *o*-catechols to allow reversible adduct formation with aryl boronic acids.[47] In non-site-selective contexts, tyrosinase has also been used for the construction of cross-linked protein hydrogels[48] and protein-modified chitosan films.[49]

To explore the potential of this enzyme in the context of the oxidative coupling chemistry, we first used abTYR to mediate the coupling of catechols to a proline engineered at the N-terminus of superfolder GFP (Pro-sfGFP, MW = 27575 Da), thereby obviating the need for large excesses of oxidants as required in previous oxidative coupling reactions. Briefly, samples containing 10 μM Pro-sfGFP and 10 equiv of 4-methylcatechol were prepared in 20 mM pH 6.5 phosphate buffer. A solution of abTYR was added at final concentrations of 83 nM or 8 nM, and the reactions were incubated for 30 min at room temperature. As a comparison, an additional reaction was run with 0.5 mM $K_3Fe(CN)_6$, as previous studies have shown that this reagent is capable of oxidizing catechols to *o*-quinones for attachment to proline N-termini.[15] High conversion to the expected product was observed using abTYR at both high and low concentrations, Figure 2, X = OH. When incubated with Pro-sfGFP alone, no background oxidation of its native tyrosines was observed, suggesting that abTYR is not capable of binding and oxidizing the endogenous tyrosines within this time frame. This result was in accordance with reports for other tyrosine-containing proteins. A survey of sfGFP substrates with other N-terminal amino acids confirmed the requirement for the proline residues, with conversions below 40% being observed with other N-terminal residues, Supporting Information Figure S1. This result was in line with previous findings for the ferricyanide-mediated version of the reaction.[15] In addition, protein sequencing of *p*-cresol-modified Pro-sfGFP after trypsin digest confirmed the proline N-terminus as the site of modification, as we have previously found, Supporting Information Figure S2.[15]

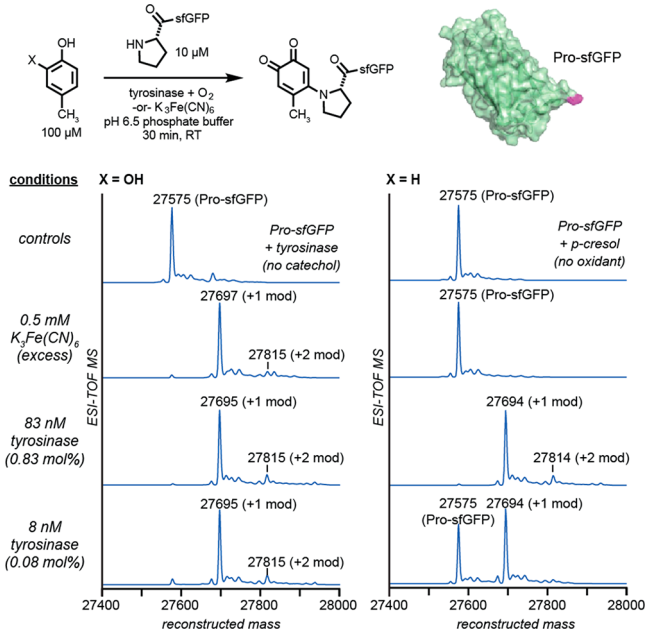

Figure 2. Site-selective oxidative coupling reactions using the tyrosinase from *Agaricus bisporus* (abTYR). Superfolder GFP with an N-terminal proline residue shown in pink (Pro-sfGFP, MW = 27575 Da) was evaluated as an initial substrate. The expected modification product ([M + H$^+$]) has a mass of 27695 Da. In some cases a small amount (<15%) of double modification can be detected at 27815 Da. TCEP was added to the $K_3Fe(CN)_6$ sample upon workup, resulting in mass increase of 2 Da due to reduction of the *o*-quinine to the catechol. Structure based on PDB ID: 2B3P.

We next explored the ability of abTYR to mediate the attachment of simple phenols to N-terminal proline residues. Phenol derivatives are easy to prepare using commercially available materials and store readily for long periods of time, thus overcoming the inherent limitations of aminophenol and catechol reagents. Samples of Pro-sfGFP were exposed to 10 equiv of *p*-cresol in the presence of abTYR under the conditions described above. After 30 min, 83 nM of abTYR successfully oxidized *p*-cresol to the *o*-quinone intermediate, completely converting the Pro-sfGFP starting material to the same product that was obtained with 4-methylcatechol, Figure 2, X = H. As anticipated, this reaction did not proceed using $K_3Fe(CN)_6$ as the oxidant. An increased dependence on abTYR concentration was observed compared to the catechol version of the reaction, but full conversion was still realized in 30 min using less than 1 mol % of enzyme relative to protein substrate.

In addition to the desired reaction product, reactions using both catechols and phenols sometimes produced a small amount (15% or less) of a double modification product. To date, we have not been able to obtain sufficient quantities of this species to characterize it using tryptic digests. As lead hypotheses, it may arise from the addition of particularly exposed lysine side chains to the quinone intermediates, or it could result from oligomerizations of the quinones through Diels–Alder reactions or other pathways.[50] If problematic, this product can be minimized by lowering the reaction temperature to 4 °C (vide infra).

Reaction Optimization for Site-Selective Biotinylation. The majority of bioconjugation reactions rely on commercially available small molecules, such as NHS-esters and maleimides, that are preactivated for attachment to lysine and cysteine residues, respectively. To make use of this convenient set of reagents, we next developed a simple protocol to convert NHS-esters to N-terminal proline specific reagents using tyramine. Biotin was chosen as an initial target for methodology development because it is commonly used to tag proteins for fluorescence labeling,[51] affinity capture,[52] and surface immobilization.[53]

To prepare biotin-phenol **1a**, a 1.1 equiv portion of the corresponding NHS-ester was added to tyramine in dry DMF, Figure 3a. The reaction was vortexed overnight at room temperature. To ensure hydrolysis of the remaining NHS ester groups, water was added before diluting the resulting phenol for use in protein coupling reactions. Portions of this solution were added to a final concentration of 100 μM to a 10 μM solution of Pro-sfGFP in 20 mM phosphate buffer at pH 6.5. A solution of abTYR was added at concentrations varying from 60 to 200 nM, and the reactions were incubated at room temperature for 30 min. At all concentrations, the enzyme successfully mediated the attachment of **1a** to the proline N-terminus of Pro-sfGFP, as indicated by ESI-TOF MS, Supporting Information Figure S3. Using 80 nM abTYR,

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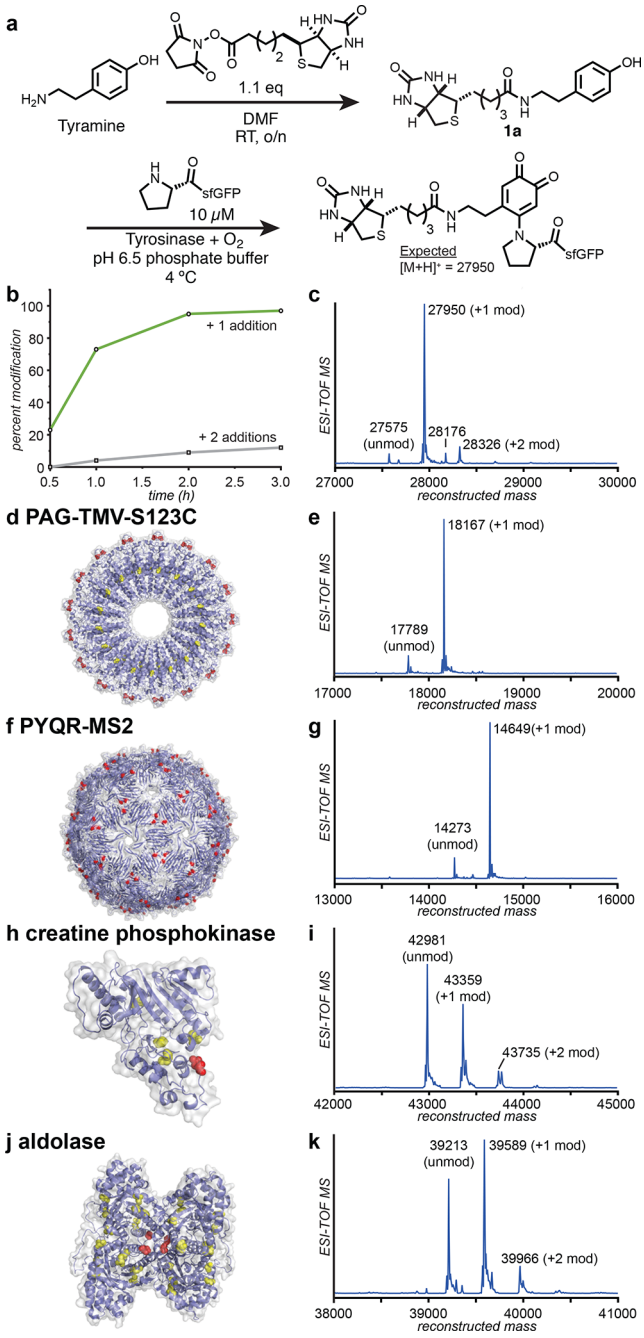

Figure 3. Site-selective biotinylation of proteins with N-terminal proline residues. (a) Using optimized conditions, biotin-phenol **1a** was attached to a variety of proteins with proline residues at the N-terminus. (b) A time course experiment for Pro-sfGFP at 4 °C showed that the reaction proceeds efficiently while minimizing overaddition product. (c) High conversion to singly biotinylated Pro-sfGFP can be achieved after 2 h at 4 °C. These conditions were used for the biotinylation of additional protein substrates with **1a**, including (d, e) TMV double disks with PAG N-terminal extensions, (f, g) MS2 viral capsids with PYQR N- terminal extensions, (h, i) commercially obtained creatine phosphokinase, and (j, k) comercially obtained aldolase. For the PAG-TMV, CPK and aldolase, the free cysteine residues (yellow) were protected with Ellman's reagent prior to the oxidative coupling reaction. The proteins were reduced with TCEP to liberate the cysteines before analysis.

~90% conversion of the starting protein was observed, and complete conversion was observed at 200 nM.

Next, we screened the effects of altered pH on the abTYR-mediated attachment of **1a** to Pro-sfGFP. A solution containing final concentrations of Pro-sfGFP at 10 μM and **1a** at 100 μM was prepared in 20 mM phosphate buffer at pH values ranging from 5 to 9. Upon addition of a final concentration of 160 nM enzyme and incubation at RT for 30 min, product formation was observed at all pH values, Supporting Information Figure S3. Yields were reduced at pH values less than 6.5, likely due to the increased protonation of the proline N-terminus. The enzyme abTYR was found to be capable of oxidizing **1a** in all buffers surveyed, Supporting Information Figure S3. To verify that no deleterious ROS species were produced during the coupling, the reaction was run in the presence of the radical scavenging agent TEMPO, Supporting Information Figure S4. Even at 1 mM TEMPO, no effect was found on the reaction progression, suggesting no appreciable amounts of ROS are involved in the tyrosinase catalyzed oxidative coupling reaction.[39,40] In addition, no adventitious oxidations of methionine or free cysteine residues[54] have been observed for the protein products.

Finally, reactions on 10 μM Pro-sfGFP in 20 mM phosphate buffer at pH 6.5 were prepared as described above with varying concentrations of phenol **1a**. After incubation with 160 nM abTYR for 30 min at room temperature, product conversion was analyzed. Complete conversion was only achieved if 100 μM (10 equiv) or greater of **1a** were used, likely because lower concentrations were too far below the K_M value of the enzyme (reported as 0.5 mM for L-tyrosine).[55] Based on these screens, the optimal conditions chosen were 10 μM protein and 100 μM **1a** with 200 nM abTYR in 20 mM phosphate buffer at pH 6.5.

During the optimization studies, we also determined the ideal storage conditions for abTYR. We found that the lyophilized powder obtained commercially could be stored at −20 °C indefinitely. For use in reactions, stock solutions were prepared at 2 mg/mL (approx 17 μM) in 50 mM phosphate buffer at pH 6.5. These solutions could be stored at −80 °C for over 100 days before use; however, storage of these solutions at −20 °C or higher temperatures led to decreases in activity over time. A summary of these studies appears in Supporting Information Figure S5.

Throughout these studies, the overmodification product was observed to varying degrees depending on the conditions employed. To decrease this unwanted product, the reaction was run at reduced temperatures. A reaction was prepared using the optimized conditions described above and was placed in a 4 °C cold room. At various times, 20 μL portions of this solution were sampled and quenched with a final concentration of 1.9 mM of both TCEP and tropolone, which reduced the activated *o*-quinone[15] and inhibited the enzyme,[40,42] respectively. Following analysis with ESI-TOF MS, complete conversion of Pro-sfGFP to the desired product was achieved in 2 h, Figure 3b and 3c. Furthermore, the lower temperature reduced the unwanted secondary modification by ~10%. The reaction is also compatible with elevated temperatures, achieving full conversion of the Pro-sfGFP starting material in only 15 min at 37 °C, albeit with increased secondary modification observed (see Supporting Information Figure S6).

It is worth nothing that this coupling reaction leads to a highly functionalized linker that could be exploited in a number of reactive contexts. As such, stability studies were carried out in various temperature and pH ranges over 24 h,

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Supporting Information Figure S7. From 4 °C to 37 °C the linkage remained intact, and over a pH range of 5 to 9 no change in the product was observed. Additional stability studies were run over 24 h in the presence of 10 mM of various nucleophiles, Supporting Information Figure S8. Biologically relevant side chains, such as the guanidinium group of arginine and the primary amine of lysine, did not lead to cleavage of the linker. In addition, anilines, an alternative nucleophile capable of reacting with *o*-quinones, and alkoxyamines, a common bioconjugation reagent, did not alter the linkage stability. Alkoxyamines were, however, found to add to the structure slowly, presumably through oxime formation.

Only free thiols are known to react appreciably with the products over time. Solutions of L-cysteine, reduced glutathione (GSH), and 2-mercaptoethanol (BME) were found to add to the initial product up to two times in locations that have not yet been characterized but are likely to be the Michael-addition sites on the *o*-quinone ring. Exposure to 10 mM cysteine, GSH, and BME also led to a small amount of cleavage of the N-terminal product (~10% for L-cys and GSH, and ~20% for BME) after 24 h, suggesting that this will have to be addressed for applications that require prolonged circulation in vivo. Products formed on proteins bearing free cysteine groups (such as those in Figure 3d,h,j) have not shown this behavior, indicating that this reactivity is limited to small thiols present in high concentrations. Thus, overall the stability studies confirm that the linkages produced by this chemistry will remain intact and unperturbed under a broad range of conditions used in most chemical biology and biomolecular materials contexts.

We also explored the efficiency of the abTYR-mediated biotinylation for other proline N-terminal proteins. Previous work from our group has shown that a K53R, K68R double mutant of the tobacco mosaic virus (TMV) capsid protein can be engineered to contain an N-terminal proline while retaining its ability to self-assemble into stable double disks.[26] This protein served as one substrate in our panel, also possessing a free cysteine group in position 123 of each monomer. Recent work from our lab and the Tullman-Ercek group has shown that the bacteriophage MS2 viral capsid can be engineered to contain proline residues at the N-termini of the coat proteins if included at the end of XXR extensions.[56,57] Finally, we examined creatine phosphokinase (CPK) and aldolase (ALD) as two commercially available enzymes that possess N-terminal proline residues.

These proteins were evaluated for site-selective biotinylation using the fully optimized reaction protocol for attachment of **1a** described above. First, the free thiols of TMV, CPK, and ALD were temporarily protected with Ellman's reagent, as we have described previously for oxidative coupling reactions.[15] Then to 10 μM of each protein in 20 mM phosphate buffer at pH 6.5 was added phenol **1a** at a final concentration of 100 μM for CPK and ALD or 200 μM for MS2 and TMV. To this was added a final concentration of 200 nM abTYR, and the reactions were run for 2 h at 4 °C. Following a 5 min quench and reduction using final concentrations of 1.9 mM TCEP and tropolone, all proteins showed conversion to the expected modified product. The addition of TCEP also had the effect of reducing the Ellman's Reagent disulfides to yield the free thiols. The engineered proline N-termini on MS2 and TMV performed better than those on CPK and ALD, likely because the extended linkers helped to increase the solvent accessibility of the proline residues.

Application of abTYR-Mediated Oxidative Coupling to a Chitin-Binding Domain. Chitin-binding domains (CBD) are protein segments capable of selectively binding chitin on the surface of yeast and pathogenic fungal cells.[58] This binding ability makes these biomolecules attractive for the targeted delivery of antifungal agents. The abTYR oxidative coupling reaction could provide a useful route to access CBD bioconjugates by targeting proline residues engineered at their N-termini, as this location is remotely disposed from the binding surface.[58] For use in these studies, a proline N-terminal variant of a CBD from the archaea *Pyrococcus furiosus* was generated.[58] This protein was expressed fused to a C-terminal thioredoxin A domain to aid in solubility and to increase the production yields. Linking the two proteins was a TEV cleavage site, allowing for future removal of the solubilizing tag, if desired, Figure 4a. For the modification of this protein, we synthesized a variety of phenol derivatives from commercially available NHS-ester precursors using the method described above, which included fluorophores with different emission wavelengths and metal-chelating agents, Figure 4, compounds **1b–d**.

The panel of phenols was coupled to 10 μM solutions of Pro-CBD-TrxA using 200 nM tyrosinase in 20 mM phosphate buffer at pH 6.5 for 2 h at 4 °C. Biotin phenol **1a** was used at 100 μM, and bulkier substrates **1b–d** were used at 400 μM. To minimize higher order modifications, some derivatives were placed in a water–ice bath, which we found better maintains the colder temperatures as the exothermic coupling proceeds. All phenolic compounds coupled readily to the proline N-terminus of the CBD construct, with little over modification observed for all compounds, Figure 4b–h.

To test the ability of the engineered Pro-CBD-TrxA construct as a fungal binding agent, we used abTYR to attach Oregon Green 488-phenol **1d** to the proline N-terminus using abTYR, yielding bioconjugate **A** in Figure 4g,h. The labeled product was obtained in high yield (~88% single addition, <5% double addition). Conjugate **A** was then incubated with *S. cerevisiae* cells for 24 h at room temperature in the absence of light (to prevent photobleaching). Analysis via flow cytometry showed that the Pro-CBD-TrxA construct with N-terminal OG488 groups retained its binding ability, Figure 4i,j. Furthermore, compared to a CBD that had been nonspecifically labeled using Oregon Green 488-NHS (see Supporting Information Figure S9), conjugate **A** showed an overall increase in the fluorescently labeled population of yeast and greater homogeneity, Figure 4j. These results highlight the ease with which phenol compounds can be used to construct site-specific proline N-terminal bioconjugates that retain proper activity.

CONCLUSION

Herein we have presented a new bond-forming strategy for the convenient site-selective labeling of bioconjugates. Using easily prepared and often commercially available NHS ester precursors, a variety of phenol derivatives of interest can be synthesized without the need for separate purification steps. These derivatives can be oxidized by a commercially available tyrosinase enzyme, after which they couple readily to proline residues introduced in the N-terminal positions of proteins. Compared to NHS-ester chemistry, this coupling achieves excellent regioselectivity, and reaction times are short even at 4 °C. Given the ease with which N-terminal proline residues can be introduced during protein expression, this method will

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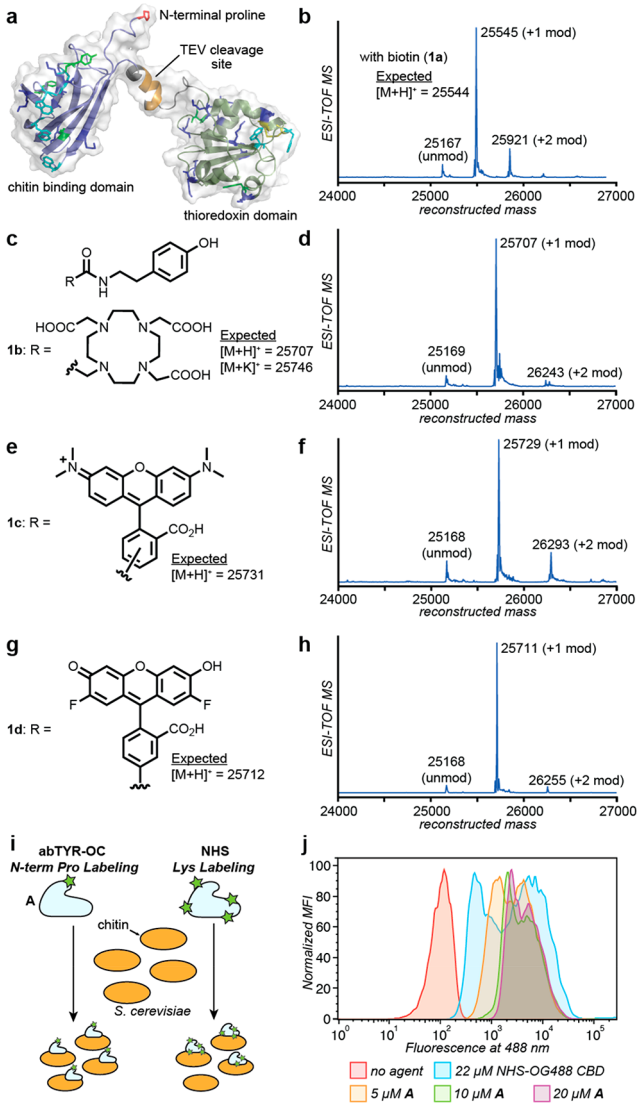

Figure 4. Tyrosinase-based labeling of a chitin binding domain (CBD). (a) A CBD−thioredoxin fusion protein was generated with an added N-terminal proline (red). Other amino acid residues shown include tryptophans (cyan), tyrosines (green), lysines (blue), and a cysteine disulfide (yellow). Structure is based on PBD IDs 2CWR and 3DBX. Using the procedures outlined in Figure 3a, the CBD construct was labeled with (b) biotin-phenol **1a**, (c, d) a DOTA-phenol, (e, f) a rhodamine-phenol, and (g, h) an Oregon Green 488-phenol. See Supporting Information for the specific conditions used in each case. (i) Yeast cells were treated with CBD-labeled **1d** using tyrosinase (A) or Oregon Green NHS at RT. (j) Flow cytometry was used to quantify the degree of binding, reported as normalized mean fluorescent intensity values.

likely be compatible with a wide range of biomolecular substrates. Finally, its reliance on low-cost and readily storable reaction precursors offers excellent potential for its use in large scale bioconjugate preparation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b10845.

Full experimental details, cloning procedures, and protein sequences (PDF)

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Notes

The authors declare no competing financial interest.

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