# Oxime Ligation via in situ Oxidation of *N*-Phenylglycinyl Peptides

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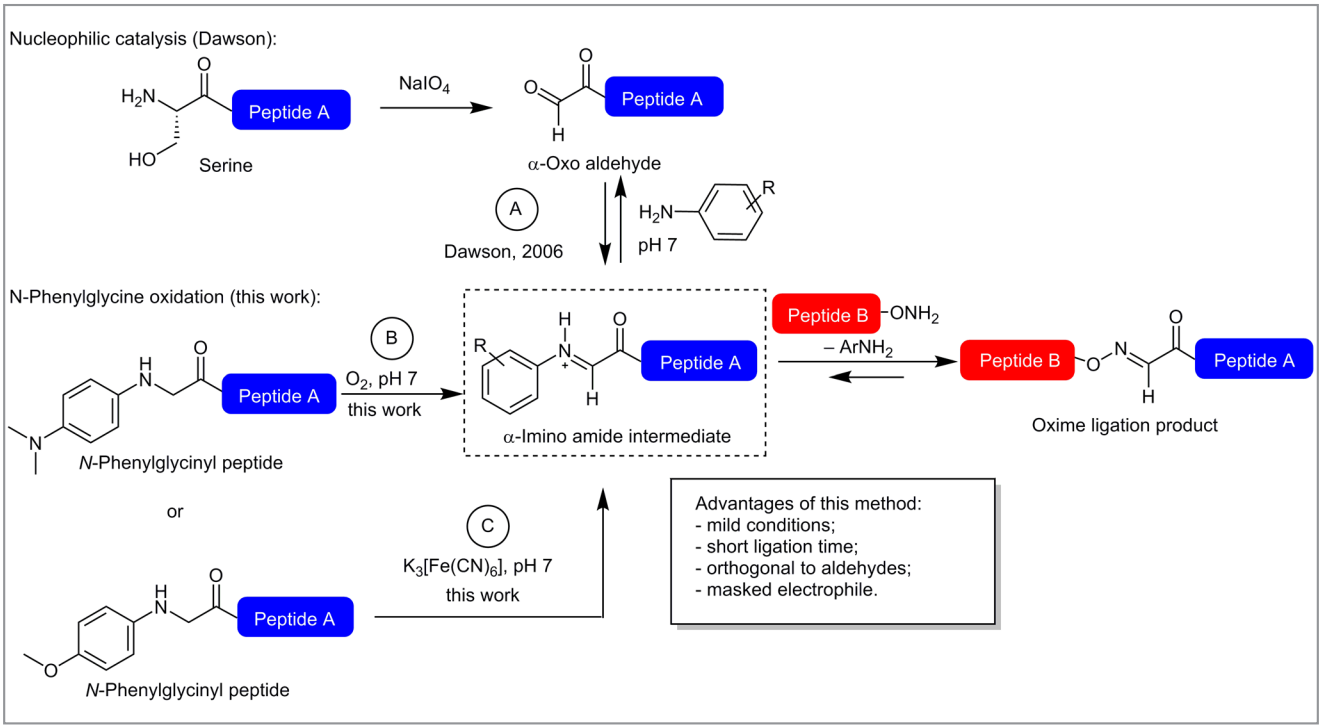
The selective formation of covalent bonds in complex biological settings requires the discovery and optimization of high-yielding bio-orthogonal chemical transformations. While many different bioconjugation reactions are available, advances in existing methods are often necessary to increase their scope and utility. In this recent publication, the group of Professor Caroline Proulx at North Carolina State University (Raleigh, USA) sought to address some of the known limitations that exist with oxime ligation reactions, where aminooxy residues react selectively with α-oxo aldehydes to form stable covalent bonds. "Pioneering work from other groups had shown that aniline Schiff base intermediates allowed reaction rate acceleration of up to 40-fold at neutral pH (Scheme 1A). This finding, along with the continued discovery of more efficient aniline catalyst derivatives, has provided significant improvements in terms of biocompatibility for oxime ligations," explained Professor Proulx. She continued: "However, we wanted to see if it was possible to bypass the use of both 1) sodium periodate (to oxidize Ser residues into α-oxo aldehydes) and 2) 10–100 mM aniline catalysts to expand the applications of oxime formation reactions. To do so, we were inspired by several reports of chemoselective oxidative coupling to *N*-phenylglycine residues, where key α-imino amide intermediates have been proposed." Notably, a recent example revealed the use of air as the only oxidant with trace amount of acid (in DCE/MeCN) to enable *N*-phenylglycine oxidation and coupling to indole and styrene derivatives (*Angew. Chem. Int. Ed.* **2014**, *53*, 13544–13547). "We hypothesized that similar mild conditions might be able to generate a reactive α-imino amide intermediate from an *N*-phenylglycine-terminated peptide in water and sought to discover precisely how acidic (what pH range) the buffer would need to be to trigger an in situ oxidation/oxime ligation reaction sequence," said Professor Proulx. She commented: "By tuning the electronics of the



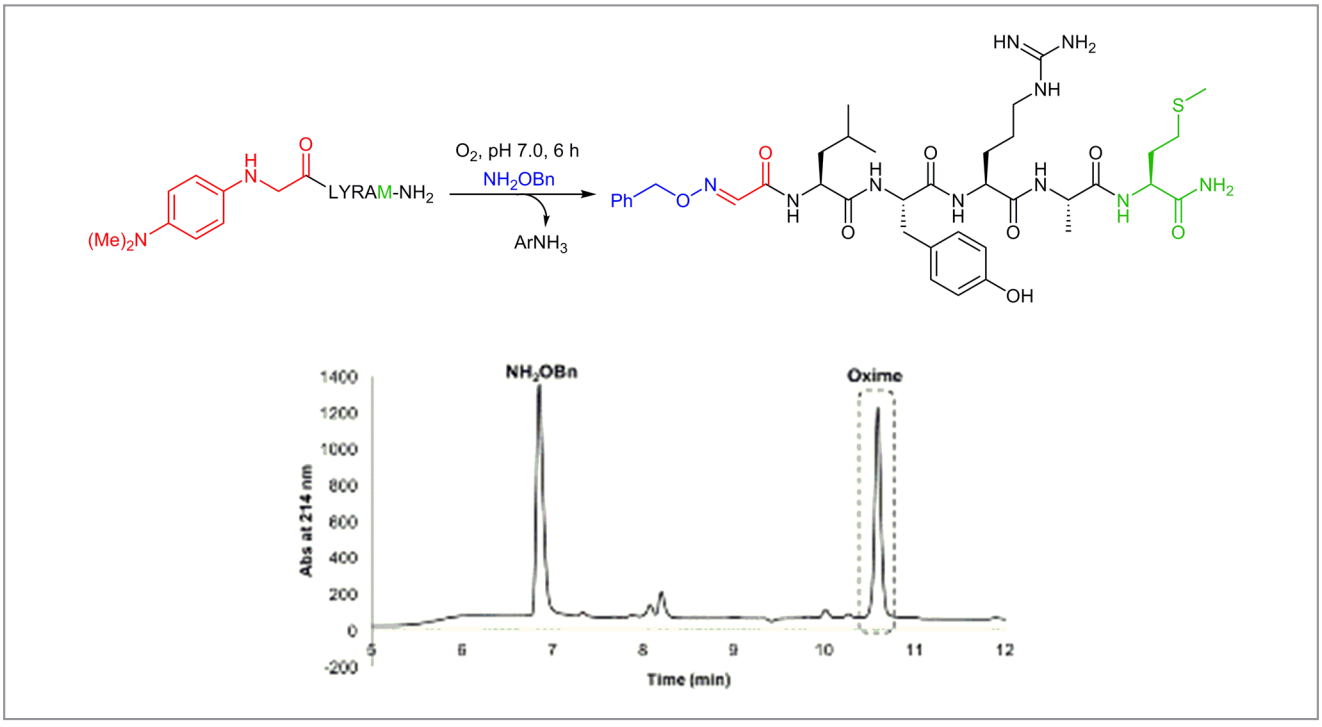

**Scheme 1** Strategies for oxime ligation reactions at pH 7.0 using A) aniline catalysis (previous work), B) in situ oxidation of an *N*-(*p*-$Me_2NC_6H_4$)glycine residue using oxygen as the only source of oxidant (this work), and C) unmasking of *N*-(*p*-$MeOC_6H_4$)glycine with potassium ferricyanide (this work)

*N*-arylglycine, we discovered that an *N*,*N*-dimethylamino substituent at the para position of the phenyl ring allowed the tandem oxidation/oxime ligation to occur at pH 7 under oxygen atmosphere (Scheme 1B, Scheme 2). In addition to this, we were curious to see if certain inert *N*-phenylglycines could be unmasked by mild oxidants such as potassium ferricyanide (Scheme 1C, Scheme 3). This proved to be the case, and a previously unreactive *N*-(*p*-$MeOC_6H_4$)glycine residue was able to undergo oxime ligation reactions at pH 7 in the presence of 1–10 mM $K_3[Fe(CN)_6]$."

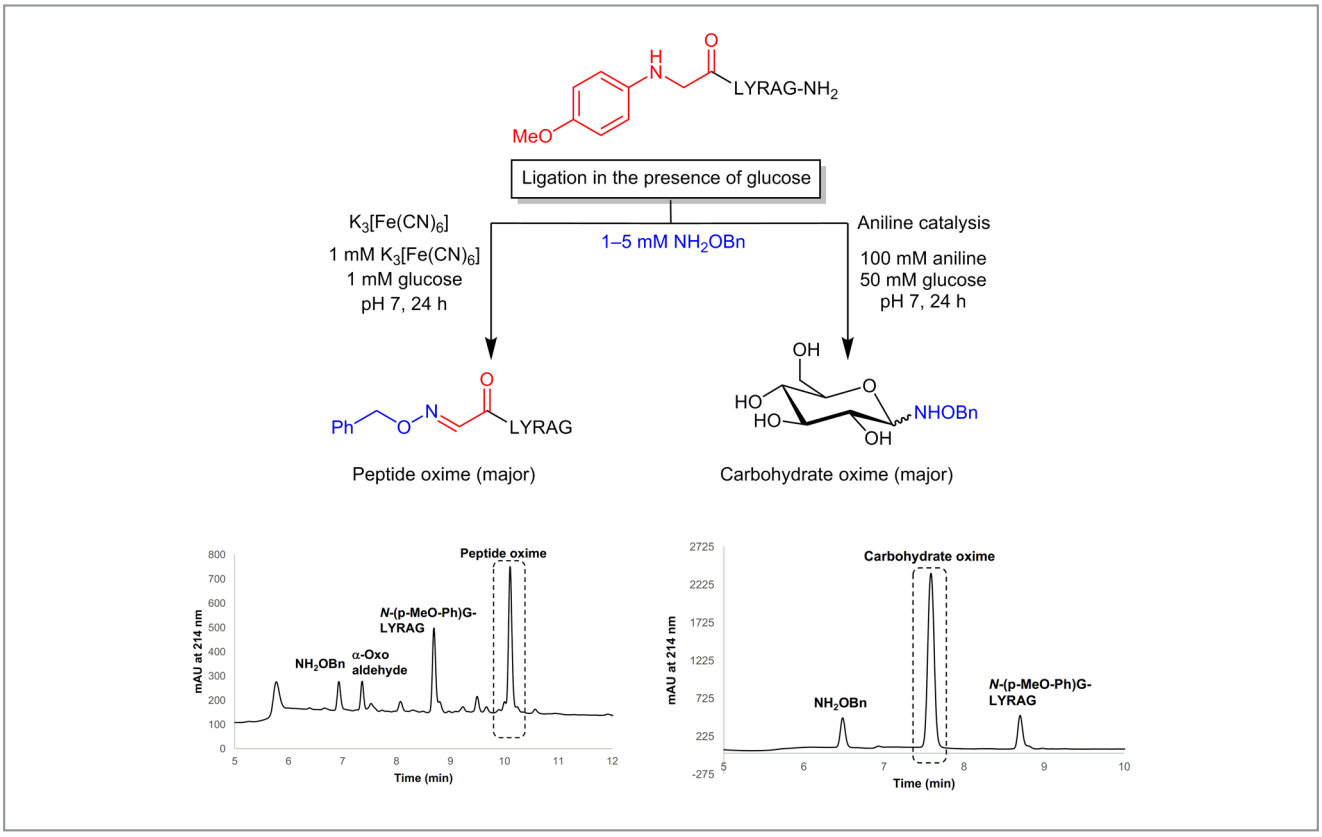
These advances in oxime ligation alleviate some of the so-called 'biorestrictions' associated with previous methods (e.g. aniline catalysts can react with other aldehydes such as carbohydrates and co-factors). However, Professor Proulx acknowledged that limitations of the group's approach include the competing hydrolysis of the reactive α-imino amide intermediate, which requires a five-fold excess of the aminooxy reaction partner, and the fact that one equivalent of aniline is still liberated during the process.

"Subjecting an *N*-(*p*-$Me_2NC_6H_4$)glycine-terminated peptide with a *C*-terminal methionine residue to our mild conditions revealed no methionine side-chain oxidation over the time period necessary for formation of the α-imino amide and oxime ligation to occur (Scheme 2)," remarked Professor Proulx. This should increase the compatibility of oxime ligation reactions using protein and peptide substrates that contain amino acids that are prone to oxidation, potentially extending beyond those included in this paper (Met, Tyr). "Alternatively, using potassium ferricyanide to selectively unmask *N*-(*p*-$Me_2NC_6H_4$)glycine, we then had a method that allowed the selective formation of the reactive Schiff base intermediate in the presence of other aldehydes," said Professor Proulx. To highlight this functional group orthogonality, the group demonstrated that oxime bond formation with *O*-benzylhydroxylamine could be selective for either their *N*-phenylgycine-terminated peptide or glucose, depending on the reaction conditions (Scheme 3).

"In the future, we hope to elucidate the mechanism of this reaction sequence and increase our understanding of the apparent relationship between substrate p$K_a$ and oxidation rate at different pH values," explained Professor Proulx. "In addition, we would like to expand the use of this chemistry beyond oxime ligation reactions to other transformations involving imine intermediates." She concluded: "Long-term goals include pursuing their unmasking potential in prodrug applications, and embedding an *N*-phenylglycine moiety as an unnatural amino acid side chain for eventual site-specific incorporation into proteins. This should increase the current




**Scheme 2** HPLC trace at 214 nm of the oxime ligation reaction between *N*-(*p*-$Me_2NC_6H_4$)G-LYRAM and $NH_2OBn$ after 6 h




**Scheme 3** Selective peptide oxime ligation in the presence of glucose and potassium ferricyanide (left) and carbohydrate oxime ligation in the presence of an *N*-phenylglycine-terminated peptide and aniline catalyst (right) with corresponding HPLC traces at 214 nm

scope and utility of oxime ligations in the toolbox of bioorthogonal ligation reactions."

## About the authors

*Prof. C. Proulx*

**Caroline Proulx** grew up in Toronto, Ontario (Canada) and obtained her Hon. B.Sc. in biopharmaceutical sciences, medicinal chemistry, from the University of Ottawa (Canada) in 2007. Subsequently, she obtained her Ph.D. in 2012 from the Université de Montréal (Canada) under the direction of Dr. William D. Lubell, where she developed submonomer methodologies for azapeptide library synthesis and applied them towards the discovery of selective CD36 receptor ligands. Following her graduate studies, she moved to Lawrence Berkeley National Laboratory (USA) from 2012–2016 as an NSERC postdoctoral fellow to study peptoid synthesis and self-assembly under the direction of Dr. Ronald N. Zuckermann at the Molecular Foundry. She began her independent career as an assistant professor at North Carolina State University (USA) in 2016, where she continues to work in the field of peptides and peptidomimetics.

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**Quibria A. E. Guthrie** grew up in Milwaukee, Wisconsin (USA) and obtained a bachelor's degree in chemistry from the University of Wisconsin – Milwaukee (USA) in 2015, with a focus in biochemistry. As an undergraduate student, she performed research under the supervision of Dr. Xiaohua Peng. She started her graduate studies at North Carolina State University (USA) in 2016 under the leadership of Dr. Caroline Proulx. Her research focus is on mild oxidation of N-phenylglycinyl peptides for bioconjugation reactions.

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