Peptide Modification and Cyclization *via* Transition-Metal Catalysis

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Abstract

Transition-metal catalysis has unlocked new paradigms for the late-stage modification and cyclization of peptides by harnessing the innate reactivity of proteinogenic amino acids. The field is rapidly evolving, with recent advances encompassing three fundamental areas—heteroatom couplings, decarboxylative cross-couplings, and C–H functionalizations—which have markedly extended the scope of conventional peptide modification and bioconjugation strategies. The advances outlined herein facilitate access to high-value peptide targets with promising applications in materials science and drug discovery.

Introduction

Unnatural amino acids are powerful building blocks for peptide-based materials and therapeutics, enabling chemists to explore beyond the structural and functional limitations imposed by the finite set of canonical amino acids. The ability to surgically tailor single amino acids within the context of a complex peptide represents an ideal approach for direct access to modified peptides, abrogating the need to prepare an orthogonally-protected, unnatural amino acid variant—an endeavor which typically requires specialist skills in organic synthesis and laborious, multi-step processes. Indeed, the advantages of late-stage modifications have prompted the recent development of several cutting-edge technologies which utilize native peptides or leverage the incorporation of select unnatural motifs to access a broad range of functionalized peptide products[1].

This opinion article will highlight a handful of transformative strategies for peptide modification and cyclization—focusing primarily on the last two years—which exploit the unique power of *transition-metals* as mediators[2-4] for covalent bond-formation or photoinduced electron transfer (PET) processes. Historically, only a small handful of amino acids have been utilized as handles for peptide functionalization[1,5,6]. However, through the strategic application of transition-metal mediators, the technologies discussed herein take advantage of discrete, often underutilized, amino acid functionalities within the context of more complex peptide and protein scaffolds. Strategies discussed fall into three main categories: 1) heteroatom couplings (at sulfur, selenium, and nitrogen); 2) decarboxylative couplings (at α-COOH, Asp, and Glu); and 3) C–H functionalizations (Figure 1). Particular emphasis will be placed on the critical role of the metal center as a reaction mediator as well as the utility of accessible peptide products. An overview of the scope and limitations of these emerging strategies is presented.

Heteroatom coupling

Several strategies for heteroatom arylation have recently been developed that exploit select side-chain functionalities as handles for palladium catalyzed C(sp^2)–X bond formation (X = S, Se, or N)[7]. Such peptide-aryl constructs are attractive given the relative stability of the linkage in comparison with other modes of bioconjugation and the commercial availability of diverse aryl substrates. Following early work employing cyclometalated gold(III) complexes[8], a pivotal study from Pentelute and Buchwald in 2015[9••] demonstrated the rapid, Cys-selective arylation of unprotected peptides using bench-stable, organopalladium reagents (e.g. **1**, Figure 2) formed through the oxidative addition of an aryl (pseudo)halide. Arylation reactions proceeded under mild, dilute conditions in mixed aqueous/organic media to form diverse peptide products, including Cys-linked drug molecules, affinity tags, and fluorescent dyes (see **2**, Figure 2A), as well as bioconjugates of native proteins and antibodies. Furthermore, the preparation of several bis-palladium species (e.g. **4**, Figure 2B) facilitated peptide macrocyclization through the dual modification of two Cys side-chains (**5**)[10]. Notably, a variety of second generation arylpalladium complexes with distinct ligands have emerged as suitable catalysts for arylation and peptide stapling at Lys residues (**6**)[11•] as well as water-soluble organometallic reagents to facilitate Cys arylation in purely aqueous media[12].

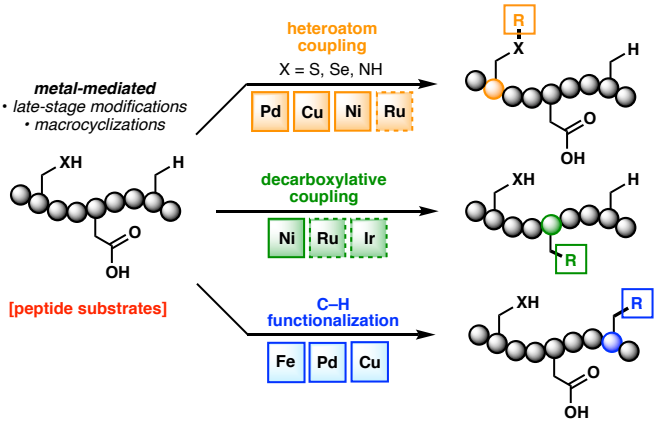

Figure 1. Transition-metal-mediated approaches to peptide modification. Representative transition-metals are highlighted, with dotted lines indicative of metals employed in photoinduced electron transfer (PET) processes.

Extending the scope of protein substrates amenable to targeted arylation, Davis and coworkers reported the use of *in situ* generated arylpalladium species for the site-selective arylation of a single Cys in mannosyl glycerate synthase (MGS), despite the presence of multiple potentially reactive Cys residues[13•].

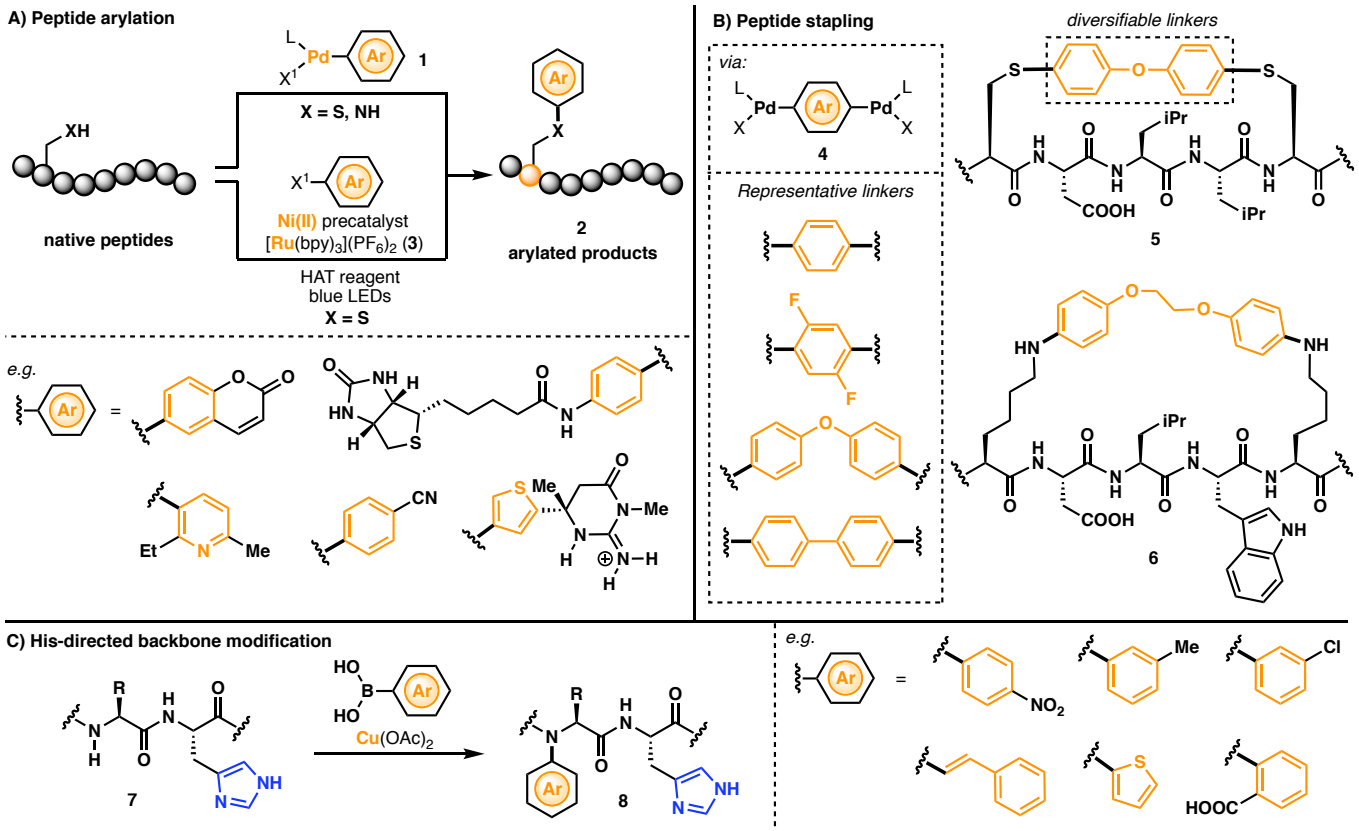

Figure 2. Metal-mediated heteroatom couplings. A) Peptide arylation using arylpalladium(II) complexes and dual nickel/ruthenium catalysis; B) Cys and Lys stapling using bis-palladium complexes **4**; C) Copper-mediated His-directed backbone arylation.

The authors cleverly exploit an endogenous metal-binding motif (Asp100-Ala101-Asp102) embedded within the protein to guide the reactive metal complex to a single Cys residue, thereby precisely incorporating a variety of functionalities, including substituted aromatics, bioorthogonal handles, and glycans.

A mechanistically distinct, dual nickel/ruthenium photoredox-catalyzed Cys arylation protocol with aryl bromides was reported by Molander and coworkers in 2018[14]. The authors utilize an ammonium *bis*(catechol) silicate hydrogen-atom transfer (HAT) reagent, which readily undergoes single electron transfer (SET) to form an alkyl radical in the presence of ruthenium photocatalyst **3** and blue light (Figure 2A). Rapid hydrogen abstraction from the thiol S–H affords a peptide thiyl radical which is relayed into the nickel-catalyzed cross-coupling cycle, ultimately affording a Cys-arylated peptide. Although less tolerant to diverse functional groups and aqueous media than the organopalladium approaches, the scalability and exceptionally low catalyst loadings (5 mol% Ni, 2 mol% Ru) render this a promising mode of Cys modification. The appeal of a radical mechanism which proceeds orthogonally to two-electron processes has also prompted the exploration of alternative, metal-free approaches to Cys arylation[15].

In a rare display of selective backbone amide modification, Ball and coworkers reported a copper-catalyzed, histidine-directed arylation of backbone N–H bonds using aryl and alkenyl boronic acids in 2016[16•]. Copper binding to internal His residues promotes site-selective backbone arylation at the neighboring *i*-1 position (see **7** to **8**, Figure 2C). Incorporation of diverse N–aryl and N–alkenyl units into peptide substrates, as well as the selective arylation of lysozyme (bearing a single His residue) were readily accomplished. Notably, the copper-mediated oxidative coupling of boronic acids to oxidized selenocysteine residues under mild, aqueous conditions has also been demonstrated—a testament to the suitability of copper-catalyzed arylation manifolds for the diverse modification of peptides[17].

Given the demonstrated versatility of heteroatom couplings, it is envisaged that such techniques will continue to emerge as valuable strategies for peptide modification. Careful tuning of the ligated-metal complex to achieve the desired reactivity without sacrificing selectivity is an important on-going challenge; nevertheless, efforts toward this goal may also deliver new functional group-selective coupling methods, targeting alternative proteinogenic handles (e.g. side-chain alcohols or amides) and building on promising new strategies for the incorporation of diverse appendages (e.g. alkynes[18]).

Decarboxylative couplings

The prevalence of carboxylic acids (including native C-terminal motifs and side-chain Asp and Glu residues) renders them ideal substrates for targeted peptide modification. Beyond the scope of dehydrogenative couplings (e.g. amidation and esterification), however, acids have been generally overlooked as handles for functionalization[1].

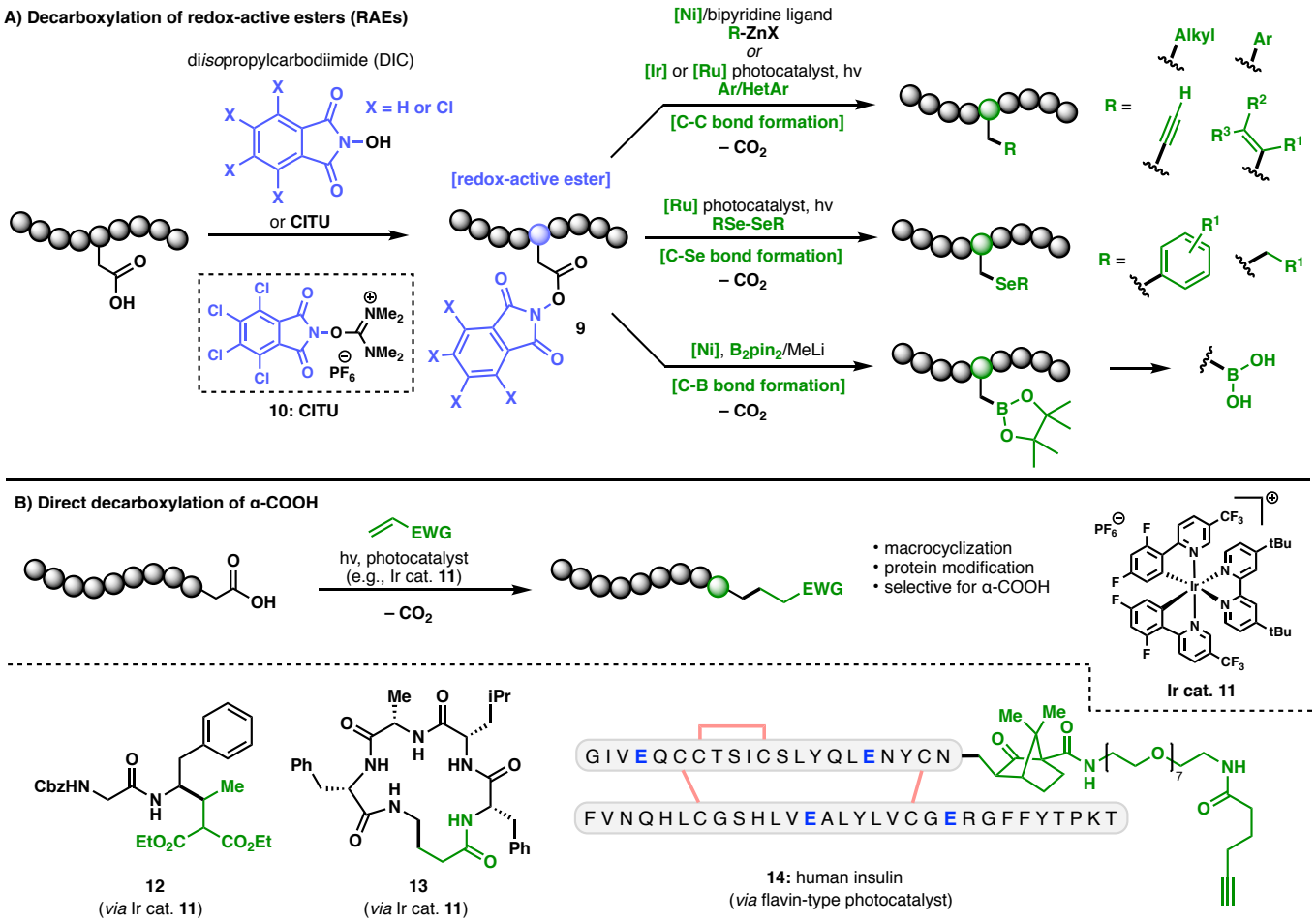

Figure 3. A) Decarboxylation of redox-active esters to forge C–C and C–heteroatom bonds; B) Direct photocatalytic decarboxylation of α-COOH in the context of peptides and proteins.

Advances in transition-metal catalyzed decarboxylative functionalizations have recently fueled a renaissance of acid-centered diversification strategies[19]. Conceptually, such methods proceed through two distinct pathways: 1) the modification of activated carboxylic acid esters (so-called redox-active esters, RAEs, such as **9**, Figure 3A); and 2) the direct modification of native acids. Both methods employ transition-metal catalysts as sources of key organometallic intermediates and/or mediators of single electron transfer (SET) to induce decarboxylative fragmentation.

Activated esters in decarboxylative functionalizations date back to Barton's seminal work on the fragmentation of thiohydroxamate esters[20] and Okada's photolytic cleavage of phthalimide esters[21]. The privileged ester substrates serve as convenient precursors to alkyl radicals, through radical fragmentation and the concomitant loss of CO_2. Renewed interest in activated esters has stemmed from the realization that alkyl radicals so-formed can be intercepted by conventional transition-metal-catalyzed cross-coupling cycles[22,23]. In 2016, the Baran laboratory reported the use of peptide *N*-hydroxyphthalimide esters as convenient precursors for on-resin, nickel-catalyzed alkyl-alkyl cross-couplings with alkylzinc reagents [24••] to afford diverse unnatural amino acids. The reactions are thought to proceed through a radical intermediate formed in the absence of light by decarboxylative fragmentation of the redox-active ester—a process instigated through SET from a suitable alkylnickel(I) catalyst. The same authors extended the scope of this C–C bond-forming methodology to include coupling with aryl, alkenyl[25], and alkynylzinc[26] reagents as well as various Michael acceptors[27], affording an array of high-value peptide targets using a combination of both solution- and solid-phase transformations (Figure 3A). Engaging the proposed radical intermediate with B_2pin_2 also provided a facile approach to peptide boronic acids[28]. Alternative, photocatalytic approaches employing iridium or ruthenium catalysts in the presence of light have facilitated strategies for arylation[29,30] and C–Se bond formation[31] that do not require the preparation of sensitive organometallic reagents. Decarboxylation is mediated by SET from the photoexcited metal catalyst, and radical trapping proceeds efficiently under mild conditions.

The need for a discrete activation step is a notable limitation of RAE-based methodologies. While new coupling reagents such as CITU **10** (Figure 3A) enable one-pot activation-coupling protocols[32], the direct decarboxylation of native acids is another attractive approach. MacMillan and coworkers have reported the decarboxylative alkylation of native, C-terminal α-carboxylic acids with various Michael acceptors catalyzed by iridium photocatalyst **11** (Figure 3B)[33-35]. First demonstrated on small peptides (e.g. **12**)[33], the protocol was later extended to peptide macrocyclization (**13**) through

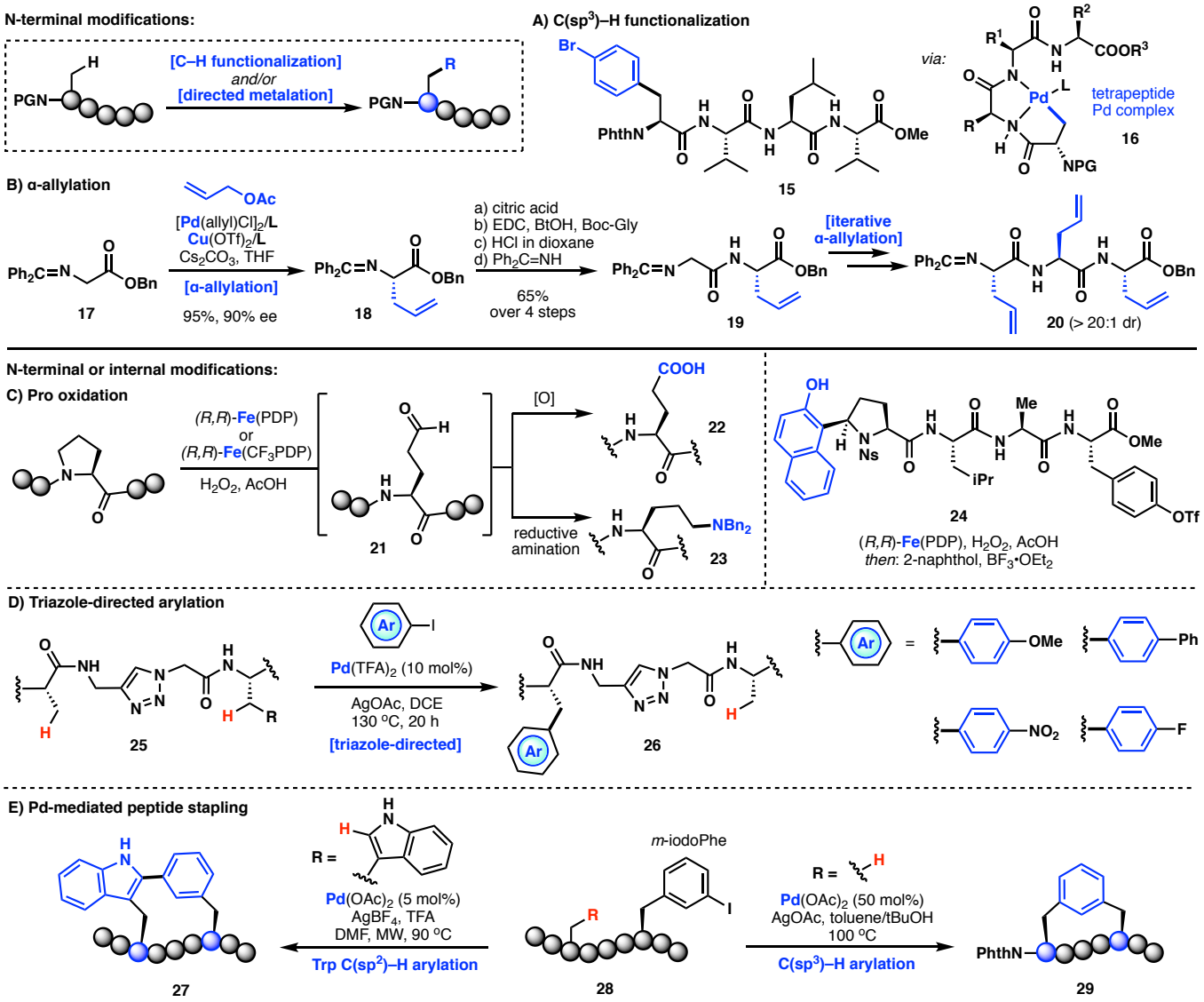

Figure 4. C–H functionalization approaches. A) Palladium-catalyzed, N-terminal C(sp^3)–H arylation; B) Dual copper/palladium α-allylation; C) Iron-mediated oxidative diversification of proline; D) Palladium-catalyzed, triazole-directed C(sp^3)–H arylation; E) Peptide stapling *via* palladium-catalyzed C(sp^2)–H and C(sp^3)–H arylation.

the intramolecular trapping of peptide radicals with appended acrylamide units[34•]. Reactions were selective for α-acids in the presence of unprotected acid side-chains—a feature which prompted examination of selective C-terminal modifications of large peptides and proteins[35•]. While the aqueous media and physiological conditions required were unsuitable for Ir-based photocatalysts, targeted modification of human insulin (**14**) with an alternative, water-soluble flavin-based photocatalyst proceeded in the presence of multiple unprotected Glu residues[35•]. Optimization of transition-metal-based photocatalytic systems may enable similar transformations in the future.

C–H functionalization and directed metalation

Strategies for the functionalization of C–H bonds represent non-traditional yet highly enabling methods for targeted peptide modification[36]. As an extension of conventional organic synthesis, such strategies are fertile "proving-grounds" for testing the chemoselectivity and broad applicability of new metal-catalyzed methods. Generally leveraging palladium catalysts, recent strategies have facilitated both N-terminal and internal amino acid modifications (Figure 4). In 2014, Yu and coworkers reported a method for the direct arylation of N-terminal Ala residues within di-, tri-, and tetrapeptide substrates (e.g. **15**, Figure 4A)[37••]. The reactions proceed in the presence of $Pd(OAc)_2$, AgOAc, and an aryl iodide, with the peptide backbone serving to direct the palladium(II) catalyst and enable activation of the Ala β-C–H bond (see **16**, Figure 4A). Although high reaction temperatures (100 °C) limit functional group tolerance, this seminal study demonstrated the vast potential for selective modifications at unactivated positions[37••].

In 2017, Zhang and coworkers reported a dual palladium/copper-catalyzed approach to the stereoselective α-allylation of N-terminal Gly and Ala residues in small peptide substrates[38]. Though not a conventional C–H functionalization approach, activation of the N-terminal amine as a Schiff base (e.g. **17**, Figure 4B) facilitated formation of a

N-metalated copper complex capable of intersecting a catalytic palladium cycle and engaging an allylpalladium intermediate to form the target α-allylated product **18**. High levels of stereoselectivity were accomplished through the judicious choice of chiral metallocene-based ligands, enabling iterative allylations to afford peptide products with excellent dr (**20**)[38].

A variety of internal modification strategies have also been reported, including the iron-catalyzed oxidative functionalization of Pro residues (Figure 4C) reported by White and coworkers in 2016[39•]. Through the intermediacy of aldehyde **21**—forged in the presence of iron catalyst, H_2O_2, and AcOH—internal Pro residues can be readily diversified (e.g., **22** and **23**). The oxidative arylation of N-terminal Pro residues with electron-rich arenes also occurs to afford diverse 5-aryl proline derivatives (**24**). The transformative power of palladium has enabled internal peptide modification and cyclization *via* $C(sp^3)$–H [40,41] and $C(sp^2)$–H functionalization[1,42-44]. Notably, Ackermann and coworkers reported in 2018 a novel strategy for C–H arylation using catalytic $Pd(TFA)_2$ and an aryl iodide in concert with an internal triazole-directing group (**25**, Figure 4D)[40]. Peptides bearing two viable sites for modification were shown to react exclusively at a single position (**26**), affording discrete arylated products. Further exemplifying the utility of C–H arylation, Albericio and coworkers have reported palladium-catalyzed protocols for peptide stapling through intramolecular arylation of the 2-position of the Trp indole ring (**27**)[43] and β-arylation of N-terminal Ala residues (**29**)[41] with an embedded *meta*-iodophenylalanine motif (**28**, Figure 4E). Applicable to both solution- and solid-phase chemistry, these methods enable the rapid and late-stage introduction of unique hydrocarbon staples. Recent manganese[45] and gold[46] catalyzed approaches to Trp alkynylation at the indole C-2 position are also promising additions to the scope of direct C–H modifications.

As a very recent trend in peptide synthesis, the potential of C–H functionalization strategies is still rapidly emerging. Current limitations, particularly for palladium-catalyzed approaches, include a reliance on elevated reaction temperatures (typically $T > 100\ ^oC$), non-aqueous media, and orthogonal protection for most non-participating functional groups. Balancing reactivity and selectivity remain challenging, but it is anticipated that a growing understanding and appreciation of the innate reactivity of proteinogenic amino acids will continue to fuel new discoveries.

Conclusions

Transition-metals have facilitated the chemical modification of peptides and proteins in myriad ways. Exploiting underutilized functional groups for structural modification and bioconjugation has opened new avenues for retrosynthetic planning, wherein carboxylic acids and even unactivated C–H bonds can serve as non-traditional "handles" for C–C bond formation, or a single N–H bond in a protein can be arylated with pinpoint accuracy. The field is rapidly evolving, with the majority of studies highlighted in this opinion article published in just the last two years. Addressing limitations in reaction scope, functional group compatibility, and the requirement for large excesses of substrate and/or metal complex will be useful topics for future investigations. In addition, increasing the simplicity and versatility of reaction protocols will facilitate more routine adoption of these methods. Given the ease with which native peptides can be prepared *via* robust—often automated—solid-phase reaction protocols, transition-metal mediated approaches to peptide modification and cyclization amenable to resin-bound substrates will prove invaluable to the advancement of the field.

Acknowledgements

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This seminal study demonstrated the applicability of bench stable arylpalladium reagents as versatile tools for rapid and selective Cys bioconjugation. Reactions proceed in aqueous media and at physiological pH to afford

functionalized peptides and proteins. Notable extensions of the methodology to facilitate peptide stapling and the preparation of a trastuzumab–vandetanib antibody-drug conjugate (ADC) are also disclosed.

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Building off of their prior work on Cys-selective arylations (see reference 9), Buchwald, Pentelute and coworkers report an approach to lysine arylation facilitated by organopalladium reagents. Careful ligand optimization enables relatively high levels of selectivity for lysine in the presence of a variety of unprotected peptide functional groups. Conjugation to natural product derivatives, drugs, chromophores, and bio-relevant tags exemplifies the utility of the method.

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Davis and coworkers take advantage of an endogenous metal-binding site within mannosyl glycerate synthase (MGS) to direct the palladium-mediated arylation of a single Cys residue in the presence of several feasible alternatives. The method allows for targeted introduction of functionalized aromatics, including glycans, and applies Cys arylation to the preparation of covalent inhibitors of MGS and the identification of metal-dependent proteins in cell lysate.

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This report outlines a copper-mediated approach to the targeted arylation of backbone amide N–H bonds in the context of unprotected peptides and proteins. Utilizing a neighboring His residue to direct copper-binding, the authors are able to modify a single backbone amide in the protein lysozyme. Given the importance of backbone modifications on the structure and function of peptides and proteins, this tool will facilitate the detailed study of select backbone analogues.

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Peptide *N*-hydroxyphthalimide (NHPI) and tetrachloro-*N*-hydroxyphthalimide (TCNHPI) esters of Asp, Glu, and α-carboxylic acids are shown to be versatile substrates for nickel-catalyzed alkyl-alkyl cross-coupling

reactions with alkylzinc reagents. The reactions proceed efficiently on resin-bound substrates by repurposing activated esters—conventional intermediates in amide bond synthesis—for C–C bond formation. This seminal study, together with subsequent variations expanding on the scope of suitable coupling partners (see references 25-28), facilitates the late-stage introduction of valuable synthetic handles.

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A diverse array of peptides bearing C-terminal carboxylic acids and appended Michael acceptors (e.g. acrylamide moieties) are shown to undergo an efficient, intramolecular Giese macrocyclization reaction facilitated by an iridium photocatalyst. The reaction is tolerant to unprotected side-chain acids and is utilized to forge cyclic peptides of varying sizes (from 11 to 47-atoms).

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The C-terminal-selective, decarboxylative alkylation of proteins is demonstrated using water-soluble, flavin-based photocatalysts. The impressive selectivity for α-COOH modification is demonstrated in the presence of peptides and proteins bearing several unprotected glutamic acid and aspartic acid residues. Notably, an organic photocatalyst, rather than a transition-metal system, was essential to the success of the reaction; this study therefore provides valuable insight into the current limitations of metal-mediated photocatalytic processes.

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This seminal study demonstrates the palladium-catalyzed C(sp^3)–H arylation of unactivated, aliphatic residues within small peptide substrates. The ability of the peptide backbone to serve as an internal directing group enables selective modification at the β-position of several N-terminal residues, including Ala, Phe, and Aib. Extension of the methodology to N-terminal acetoxylation is readily accomplished in the presence of acetic anhydride. Moreover, the conceptual advance outlined in this article—namely the suitability of unactivated C–H bonds as handles for diverse peptide modification—marks a paradigm shift in peptide retrosynthetic analysis.

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The diverse, oxidative functionalization of N-terminal and internal Pro residues mediated by an iron catalyst in the presence of H_2O_2 and AcOH is disclosed. 5-

hydroxyproline and its ring-opened aldehyde tautomer formed following oxidation at the C5 position of Pro are versatile intermediates for subsequent functionalizations. Arylation with electron-rich arenes, further oxidation, reductive amination, and Wittig olefination provide access to diverse peptide products from a single precursor. In addition to the late-stage tailoring of Pro-containing peptides, the catalytic system was also amenable to the C–H hydroxylation of Leu and Val side-chains.

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