Biaryl bond formation through biocatalytic oxidative cross-coupling reactions

Lara E. Zetzsche[#], Jessica A. Yazarians[#], Suman Chakrabarty, Meagan E. Hinze,
April L. Lukowski, Leo A. Joyce, and Alison R. H. Narayan*

Abstract | Biocatalysis offers compelling advantages in synthesis, often becoming the method of choice based on sustainability, safety, and selectivity considerations. Despite these advantages, enzymes in synthesis are typically dedicated to functional group interconversions in linear synthetic sequences and have not been broadly integrated into the retrosynthetic logic for carbon skeleton assembly. In this article, we disclose a biocatalytic platform for fragment coupling to assemble target molecules convergently. Specifically, we report a strategy for biocatalytic phenolic cross-coupling through oxidative C–C bond formation. Using cytochrome P450 enzymes, we demonstrate the ability to catalyze cross-coupling reactions on a panel of phenolic substrates and further demonstrate the ability to tune these catalysts to possess the desired reactivity, site-, and atroposelectivity. This streamlined method for constructing sterically-hindered biaryl bonds provides an engineerable platform for assembling molecules with programmable catalyst-controlled reactivity and selectivity unprecedented with small molecule catalysts.

Convergent synthetic strategies enable the efficient construction of carbon frameworks, quickly generating complexity by stitching individual building blocks together.¹ Chemists depend on transformations, such as cross-coupling reactions, that can reliably be programmed into synthetic routes for convergent approaches.² Ideally, reactions planned for the assembly phase of a convergent synthesis are both perfectly selective and tolerate a breadth of functional groups to minimize the production of undesired products, installation of protecting groups, or unnecessary redox manipulations.³ These qualities are common in biocatalytic reactions due to the catalyst-controlled selectivity possible with large molecule catalysts;⁴ however, the enzymatic transformations most commonly applied in synthesis are confined to functional group interconversions, not convergent steps within a synthetic route.^{5–11} To enable convergent biocatalytic strategies, a handful of biocatalytic methods have recently been developed,^{12–15} foreshadowing the potential of convergent biocatalysis in synthesis. Based on the paucity of enzyme-mediated methods for uniting substantial fragments, we developed an intense interest in expanding the repertoire of biocatalysts capable of convergent fragment couplings. As a first step toward this goal, we sought to develop a platform for the biocatalytic assembly of carbon frameworks through oxidative cross-coupling reactions, thus providing a solution for this transformation's outstanding reactivity and selectivity challenges through biocatalysis and enabling convergent synthetic routes (Figure 1a).

We chose to target biaryl bond formation as a model transformation for convergent cross-coupling reactions, given the ubiquitous nature of biaryl scaffolds in drugs, materials, and ligands for asymmetric catalysis (Figure 1b) and the fundamental synthetic challenges presented by the selective construction of these core structures (Figure 1c).^{16,17} The most commonly employed strategy to construct these molecules relies on metal-catalyzed cross-coupling reactions such as Kumada, Negishi, Stille, and Suzuki cross-coupling reactions.^{2,18–20} These reactions share the advantage of preprogrammed site-selectivity, albeit at the expense of prefunctionalized aryl fragments, adding extra steps in a synthetic campaign.²¹ Additionally, these reactions are often limited in functional group tolerance, can require extensive screening to identify suitable conditions for each new pair of coupling partners, and are still restricted in the connectivities accessible,^{20,22–27} with tetra-*ortho*-substituted couplings remaining an outstanding challenge.^{28,29}

Oxidative coupling provides an attractive strategy for convergent coupling through the net transformation of two C–H bonds into a C–C bond.^{21,30,31} However, these advantages often come at the

expense of the efficiency and selectivity of this transformation, which is typically dictated by the intrinsic stereoelectronic properties of each substrate, thereby limiting the versatility of this transformation and hindering its routine incorporation in synthetic strategies (Figure 1c).^{21,32-34} Numerous metal catalysts and metal-free oxidants have been reported for the dimerization and cross-coupling of phenolic substrates;

Fig. 1 | Biocatalytic convergent fragment coupling reactions. **a**, Biocatalysts are traditionally employed in the synthesis of chiral building blocks or the late-stage functionalization of complex molecules. We sought to expand the utility of biocatalysts to convergent fragment coupling reactions, thereby merging the selectivity provided by biocatalysts with the efficiency of convergent synthesis to enable the biocatalytic construction of carbon frameworks. **b**, Biaryl bond formation is targeted as the model transformation for fragment coupling given the privileged nature of biaryl scaffolds in drug discovery and asymmetric catalysis. **c**, Chemical methods for oxidative coupling reactions face fundamental challenges in reactivity and selectivity. **d**, In this work, we report the first biocatalytic platform for the convergent fragment coupling of phenolic substrates through oxidative C–C bond formation.

however, the application of these methods is commonly restricted to electron-rich phenols or those bearing only mildly electron-withdrawing groups such as halides.³²⁻⁴⁹ When general reactivity can be achieved, controlling the selectivity of the oxidative coupling reaction presents several additional layers of difficulty with the need to exert control over chemo-, site-, and atroposelectivity to form sterically hindered biaryl bonds (Figure 1c). Extensive investigation over the last two decades has provided insight into the selectivity outcomes of phenolic oxidative cross-couplings, leading to the development of computational tools and predictive models based on mechanistic investigation of select catalysts and the electronics of each phenol to better understand the chemoselectivity outcomes of these reactions.^{33,34,37} Additionally, methods to control the site- and atroposelectivity have been reported, but considerable hurdles remain in generating a given product isomer, especially when the intrinsic steric and electronic properties of the reacting phenols preclude access to the desired product isomer.^{33,34,44,50-52}

Biocatalytic oxidative cross-coupling reactions have the potential to overcome these limitations inherent to established methods for direct oxidative coupling by providing a paradigm with catalyst-controlled selectivity. Nature has evolved catalysts for oxidative dimerization of phenolic compounds to generate biaryl natural products.^{53,54} Whereas a growing number of these catalysts have been identified in biosynthetic pathways of biaryl natural products (Supplemental Table S2),⁵⁵⁻⁶⁸ a much more vast pool of related sequences remains underexplored with untapped potential to catalyze a diverse array of oxidative coupling reactions (see sequence similarity network, SSN, in Figure 1d). Both laccases and cytochrome P450 monooxygenases (P450s) are known to carry out this transformation.⁹ Although laccases have been employed in biocatalysis, these enzymes have only been shown to exert control over the selectivity of the C–C bond-forming event following the initial oxidation in a limited number of examples,^{68,69} as laccases more commonly afford complex product mixtures.^{70,71} In contrast, P450s associated with secondary metabolite biosynthesis often exhibit excellent control over the site- and atroposelectivity in oxidative coupling reactions.⁷² Among these, the most well-studied P450s perform intramolecular biaryl bond-forming reactions and often require carrier protein-bound substrates,^{73,74} limiting their potential application as convergent biocatalysts. Promisingly, P450s that mediate intermolecular oxidative dimerization chemistry have recently been identified but have not been investigated as biocatalysts capable of chemistry beyond their native reactivity.⁵⁵⁻⁶⁵ We hypothesized that this class of enzymes could be developed into tunable biocatalysts for convergent fragment coupling, providing a paradigm for oxidative cross-coupling reactions with catalyst-controlled selectivity.

With the complementary reactivity and selectivity of a growing set of wild-type P450s providing a robust starting point, we envisioned developing a biocatalytic method for the site- and atroposelective oxidative cross-coupling to provide a solution to this holy grail reaction. Among the P450s previously characterized to perform oxidative dimerization reactions in the biosynthesis of biaryl natural products, we were initially drawn to a small cluster of *Aspergillus* P450s that each imparts a unique site-selectivity in the oxidative dimerization of coumarin **4**, as demonstrated by Müller and coworkers (Supplemental Table S2 and Figure S27).^{55,58} This catalyst-controlled site-selectivity provided us a tantalizing starting point for developing an enzymatic platform for oxidative coupling reactions.

To assess the selectivity and scalability of this enzymatic reaction, we conducted analytical scale biotransformations in *Saccharomyces cerevisiae* heterologously expressing KtnC.⁵⁸ We observed the formation of the expected dimeric product **7**; however, the amount of enzyme produced and low substrate loading in *S. cerevisiae* was not amenable to preparative scale reactions. To develop a more scalable whole-cell biocatalytic platform in yeast, the gene encoding KtnC was incorporated into the genome of *Pichia pastoris*, an alternative yeast strain known for high levels of recombinant protein production.⁷⁵ This new biocatalytic system gave significantly increased quantities of heterologously expressed protein and allowed for higher substrate loading, providing up to a 3.4-fold increase in the total formation of biaryl

product (Supplemental Figure S28).^{75,76} Ultimately, the development of this whole-cell biocatalytic system provided us with a platform for the rapid profiling of KtnC reactivity with analytical scale reactions in 24- and 96-well plates, as well as preparative scale reactions with up to 100 mg substrate per liter of culture for the isolation of biocatalytic products (Supplemental Section VI).

Fig. 2 | Scope of oxidative cross-coupling reactions catalyzed by wild-type P450 KtnC. **a**, KtnC displayed promiscuity in the cross-coupling of native coumarin **4** with a range of non-equivalent coumarins with varying steric and electronic properties. **b**, Increasing the steric bulk of the ester at the C4 position of the coumarin substrate was used as a handle to diverge from the native 8,8' site-selectivity to instead access synthetically challenging 6,6' cross-coupled products. **c**, The scope of the cross-coupling reactions could be expanded to non-coumarin phenols, albeit with a decrease in the reactivity and selectivity as the substrate structures diverged further from the native coumarin scaffold. General reaction conditions: *P. pastoris* cells expressing KtnC in the presence of **4** and **5** (1:10 molar ratio with 25–100 μ M **4**) at 30 °C with shaking at 235 rpm for 48–72 h. Cross-couplings reported as percent conversions or (*) percent yields.

With a system amenable to both analytical and preparative scale reactions in place, we sought to move beyond the dimerization reactions, for which nature has designed these catalysts, to develop a general platform for oxidative phenolic cross-coupling reactions. We hypothesized that the co-localization of two non-equivalent phenolic substrates in the enzyme active site could provide a route toward cross-coupled products in which selectivity is dependent on substrate localization and positioning in the active site, rather than the inherent electronic properties of each substrate. In a proof-of-concept experiment, coumarins **4** and **16** were both added to cultures expressing KtnC. In this experiment, minor amounts of cross-coupled products were detected along with dimer **7**. The yield of the cross-coupled product could be enhanced by increasing the concentration of the coupling partner relative to substrate **4** (Supplemental Figure S30). Using this strategy for KtnC-catalyzed cross-couplings, coumarin substrate **4** was paired with a range of phenolic substrates to form cross-coupled products (Figure 2). Specifically, we observed substrate promiscuity in KtnC-catalyzed cross-coupling reactions with a panel of coumarin substrates (Figure 2a). Initial cross-coupling reactions between **4** and coumarins **9–12** demonstrated that maintaining similar substitution patterns to **4** at the C4 and C5 positions of the coumarin is beneficial but not required for activity. Additionally, KtnC tolerated a range of electron-rich and electron-deficient substituents (**13–16**), diverging from the electronic restrictions typically hindering small molecule-catalyzed oxidative cross-couplings.^{33,34} Added steric bulk at the C4 and C5 positions of coumarins **17–22** was also readily accepted. In contrast to the high levels of reactivity achieved in coumarin cross-couplings with KtnC, modest to no cross-coupling reactivity was observed when we subjected coumarins to established methods for the oxidative coupling of phenols (Supplemental Table S1).^{31,37,50,77–81} In a model cross-coupling reaction between coumarins **4** and **18**, the most productive chemical cross-coupling gave a 23% conversion with vanadium oxyfluoride compared to an 87% conversion with KtnC.⁸² Moreover, the vanadium-catalyzed reaction resulted in an inseparable mixture of isomeric coumarin–coumarin cross-coupled products and dimers (Supplemental Figure S14).

To investigate the selectivity of KtnC mediated cross-coupling, we fully characterized both the site- and atroposelectivity for a suite of coumarin pairs. In reactions with coumarins harboring increased steric bulk at the C4 vinylogous ester (see **18–22**), we observed the formation of a second cross-coupled product in addition to the expected 8,8'-product (Figure 2b). For example, in the reaction between **4** and ethyl ester-bearing **18**, two products were formed in an 8:1 ratio, with the major product harboring the 8,8'-connectivity (**23**) and the minor product possessing an unexpected 6,6'-connectivity (**24**). As the steric bulk of the coumarin coupling partner was further increased to a butyl ester (see **22**), the site-selectivity of the reaction was altered, giving a 2:5 ratio of the 8,8'-product (**25**) to the 6,6'-product (**26**). The atroposelectivity in the formation of the 8,8'-products was excellent (see **7**, **23**, and **25**), whereas the formation of the 6,6'-products with increased steric bulk showed erosion of atroposelectivity (see **24** and **26**). Notably, both prefunctionalization and direct oxidative coupling synthetic strategies were unsuccessful in generating the 6,6'-isomer in these coumarin–coumarin cross-coupling reactions, highlighting the electronic biases that impede access to a specific connectivity using small molecule-based strategies (Supplemental Figure S8 and S10).^{33,34}

In addition to forming a range of unsymmetrical bicoumarin products, a panel of more diverse phenols were cross-coupled to coumarin **4** (Figure 2c). In general, we observed superior reactivity with phenolic substrates that more closely resembled the native substrate (**4**), such as benzofuran **27** which afforded a 52% conversion to cross-coupled product. Additionally, naphthol **28** which possesses similar stereoelectronic properties to coumarin **4** was substantially more reactive than 2-naphthol (**31**), with conversions of 20% and 2% to cross-coupled products, respectively. We hypothesized that the limitations in substrate scope could be overcome and the versatility of the biocatalytic platform further expanded by increasing the number of catalysts at our disposal. To do this, we invoked a multipronged strategy in which

we both profiled the oxidative cross-coupling reactivity of a different wild-type P450 enzyme (Figure 3) and engineered a series of novel P450 variants with improved activity and selectivity in a target cross-coupling reaction (Figure 4).

To identify an alternative P450 catalyst with complementary substrate scope to KtnC, we took a bioinformatics-guided approach using SSNs as a tool to visualize the natural sequence space for P450s. We hypothesized that SSN-analysis of P450s known to catalyze oxidative dimerization reactions within the larger P450 sequence space would allow us to deliberately select an enzyme with a high likelihood of having complementary reactivity to KtnC. We first constructed an SSN with the entire P450 protein family (Supplemental Figure S26), then truncated the dataset to edges possessing an alignment score greater than 110 with a P450 known to oxidatively dimerize phenols (Figure 1d).⁸³⁻⁸⁶ We observed that characterized P450s that clustered together (indicating high levels of sequence similarity) functioned on similar substrates, albeit with divergent site-selectivity. In contrast, characterized enzymes in different clusters typically functioned on different substrate scaffolds (Supplemental Figure S27). Based on these trends, we selected CYP158A2 as a potentially complementary catalyst to KtnC due to its native reactivity in the dimerization of flaviolin (**32**),^{63,87,88} a substrate harboring a naphthoquinone core that differed structurally and electronically to the coumarin core present in **4**. Moreover, we anticipated that developing a platform for the investigation of CYP158A2 would provide a promising starting point for the broader investigation of fragment coupling chemistry within bacterial P450s due to its presence in a cluster of over 500 other bacterial P450 sequences with unknown functions (Figure 3a).

Fig. 3 | Exploring alternative sequence space for catalyst with complementary reactivity. **a**, SSNs are powerful tools to visualize the natural sequence space of a type of enzyme. To examine the complementarity of reactivity across P450 clusters, we selected the biflaviolin synthase CYP158A2 as a representative catalyst within a much larger pool of related bacterial P450 sequences. **b**, An engineered bacterial CYP158A2 fusion enzyme (A2-RhFRed) catalyzes cross-couplings with naphthols, demonstrating complementary reactivity to KtnC. General reaction conditions: 10 μ M A2-RhFRed in the presence of 5 mM glucose-6-phosphate, 1 mM NADP⁺, 1 U/mL glucose-6-phosphate dehydrogenase, 100 μ M **5**, and 300 μ M **33** in tricine buffer pH 8.3 at 30 °C for 16 h; cross-couplings reported as percent yields for reactions in which authentic cross-coupled products were chemical accessible, otherwise relative percent conversions (*) are reported.

In order to profile the cross-coupling reactivity of CYP158A2, we first engineered the type I bacterial P450 into an artificial fusion enzyme by tethering the P450_{RhF} reductase domain (RhFRed) to the C-terminus of CYP158A2 to reconstitute the required electron transport for catalytic activity (Supplemental Figure S29).⁸⁹ We were most interested to see if A2-RhFRed would productively catalyze oxidative cross-coupling reactions with naphthols (see **33**) to provide us with a complementary catalyst to KtnC. Upon heterologous expression and purification of the engineered fusion enzyme from *E. coli*, the cross-coupling between coumarin **10** and 2-naphthol (**31**) proceeded with a 13% yield, a five-fold improvement in yield compared to the same cross-coupling catalyzed by wild-type KtnC. Furthermore, we observed that A2-RhFRed productively cross-coupled a diverse panel of phenolic substrates (**5**) to a naphthol partner (**33**) with low to moderate yields (Figure 3b). Notably, in our hands oxidative cross-couplings mediated by small molecule-based methods only provided access to a portion of the cross-coupled products A2-RhFRed formed, with both general reactivity and competing dimerization representing a significant hurdle in small molecule-mediated reactions (Supplemental Table S1 and Figure S1). Furthermore, the majority of these A2-RhFRed reactions were predominately selective for a single cross-coupled constitutional isomer (see **38–44**); however, A2-RhFRed exerted little control over atroposelectivity, with only naphthol–naphthol cross-coupled products **43** and **44** being formed with slight enrichment of one atropisomer over the other (Supplemental Figure S80). Altogether, these results demonstrate the complementary reactivity achievable with different P450 catalysts and expand the scope of biocatalytic cross-couplings within this platform to naphthol cross-couplings.

Having demonstrated the diversity of cross-couplings achievable within this biocatalytic platform using two different wild-type P450s, we sought to explore the tunability of these enzymes through protein engineering.⁹⁰ Specifically, we anticipated that protein engineering could address critical limitations in reactivity and selectivity displayed in unnatural cross-coupling reactions with the wild-type enzymes. Toward this goal, we targeted the cross-coupling catalyzed by wild-type KtnC between coumarin **10** and 2-naphthol (**31**) as a model system due to its low levels of cross-coupling activity (<3% conversion) and lack of site-selectivity, resulting in a complex mixture of cross-coupled and dimeric products (Figure 4a and Supplemental Figure S82). We envisioned that the successful engineering of a catalyst that could transform this low-yielding, unselective biocatalytic reaction to a synthetically viable method for the preparative scale synthesis of cross-coupled product **34** would clearly demonstrate the potential of this platform for target-oriented synthesis. Additionally, this reaction created an opportunity to test the limits on engineering these enzymes for both the desired activity and multiple forms of selectivity.

To overcome the challenges in the target cross-coupling reaction, we developed a high-throughput directed evolution strategy that would identify P450 variants with both improved activity and selectivity for the target reaction (Figure 4b). Enzyme libraries were generated through semi-rational mutagenesis, wherein beneficial mutations were identified through site-scanning saturation mutagenesis of active site residues and then folded together in combinatorial libraries made by multiple site-directed mutagenesis.⁹¹ This mutagenesis strategy generated thousands of variants in each round of evolution, with each variant harboring one or several mutations within 12 Å of the predicted substrate-binding region in the active site. Reactions with these engineered protein libraries were performed as yeast biotransformations in 96-well plates and analyzed for total cross-coupled product formation by high-throughput RapidFire mass spectrometry. Reaction mixtures containing higher levels of cross-coupled product formation were then analyzed for site-selectivity in a second-tier screen using standard LC-MS. Ultimately, this two-tier mass spectrometry screen enabled rapid identification of variants with improved activity and selectivity for the target cross-coupling reaction.

Through iterative rounds of directed evolution, we progressively improved the total activity and site-selectivity in the desired biocatalytic cross-coupling reaction (Figure 4c). Initially, we targeted

improving the total cross-coupling activity, exclusively increasing the formation of cross-coupled products 19-fold over the first two rounds of evolution while keeping the competing levels of coumarin dimerization low. However, the site-selectivity of the reaction was undesirable, with the C–O cross-coupled product **37** representing nearly half of all the cross-coupled products formed by LxC2. In subsequent engineering rounds, we targeted the identification of mutations that improved site-selectivity in addition to activity, leading to the identification of two mutations (P142R and R401Q) that switched

Fig. 4 | Engineering P450 biocatalysts for improved activity and selectivity. **a**, Target reaction selected for directed evolution campaign, with wild-type KtnC catalyzing the cross-coupling at a 2.7% yield to four different cross-coupled isomers, in addition to the competing dimerization. **b**, Strategy for the directed evolution of P450s for improved activity and site-selectivity employing semi-rational mutagenesis and a two-tier mass spectrometry screen, as represented by data from round three of evolution above. **c**, Over five rounds of evolution, a total of nine active-site mutations were incorporated to provide LxC5 with 92-fold improvement in activity and site-selectivity toward the target cross-coupled product **34**. **d**, In addition to site-selectivity, we demonstrated that the atroposelectivity of the reaction was tunable with active site mutations.

the major site-selectivity to the desired 8,1'-product **34**. Ultimately, through five rounds of evolution, we engineered a biocatalyst with 92-fold improvement in activity to form the target cross-coupled product **34**, from an initial 0.5% yield catalyzed by the wild-type enzyme to a 47% yield catalyzed by LxC5 on an analytical scale. We subsequently carried out preparative scale biotransformation using LxC5 and isolated the cross-coupling product **34** in a 50% yield (Supplemental Section VI). The efficiency of this reaction was further exemplified in comparison to the chemical synthesis of **34**, with a direct oxidative cross-coupling strategy using a copper catalyst giving a 19% yield (Supplemental Table S1, Entry 2a)^{77,78} and a Suzuki cross-coupling strategy providing a 26% yield over four steps (Supplemental Scheme S3).

In our engineering campaign, we focused primarily on the reactivity and the site-selectivity of the cross-coupling reaction. Through the progress of evolution, the atroposelectivity of the reaction decreased from an 80:20 er with the wild-type enzyme to a 55:45 er with LxC5 (Supplemental Figure S87). We next leveraged the engineering platform to tune the atroposelectivity of the cross-coupling reaction. In this regard, we screened a multiple site-directed mutagenesis library combining mutations identified throughout the evolution process that were beneficial for atroposelectivity and reversed mutations that were detrimental to atroposelectivity. In a proof-of-concept screen, we identified several variants with improved atroposelectivity including LxC6, which formed **34** with 75:25 er (Figure 4d and Supplemental Table S7). These results demonstrate that this biocatalytic platform is highly engineerable for a desired activity, site-selectivity, and atroposelectivity in cross-coupling reactions, thereby providing a programmable platform for catalyst-controlled oxidative cross-coupling reactions.

The challenge of overriding innate reactivity and selectivity in oxidative coupling reactions has limited the reliable use of this method for synthetic convergence. Here, a biocatalytic solution overcomes reactivity hurdles in an oxidative cross-coupling reaction by surpassing electronic limitations and redefining the substrates that can be efficiently cross-coupled. Further, using a large molecule ligand provides the opportunity to achieve catalyst-controlled site- and atroposelectivity in biaryl coupling reactions. The wealth of natural catalysts available, along with the demonstrated ability to optimize enzymes for the desired substrate, yield, and selectivity through protein engineering, positions this catalyst-controlled oxidative coupling approach to be a powerful tool for synthetic chemists to enable convergent assembly of molecules.

Author Contributions L.E.Z., J.A.Y., S.C., M.E.H., and A.R.H.N. designed experiments and wrote the manuscript with feedback from all authors. L.E.Z. and A.L.L. generated yeast strains used in this work. J.A.Y., S.C., and M.E.H. synthesized substrates and racemic product standards. L.E.Z., J.A.Y., and M.E.H. conducted biocatalytic reactions. L.E.Z. performed the protein engineering presented in this work. L.A.J. calculated CD spectra and assigned the absolute configuration of products.

Acknowledgements This research was supported by funds from the University of Michigan Life Sciences Institute, the University of Michigan Chemistry Department, the Alfred P. Sloan Foundation, and the Research Corporation Cottrell Scholars program. Initial studies on the selectivity of dimerization reactions were supported by the National Institutes of Health R35 GM124880, and protein engineering (rounds 4-6) were supported by generous funds from the Novartis Global Scholars Program. L.E.Z. is grateful to the National Institutes of Health National Center for Complementary & Integrative Health of the National Institutes of Health F31AT010973, J.A.Y. thanks the National Science Foundation GRFP, A.L.L. acknowledges the Rackham Graduate School (UM) and the National Institutes of Health F31 NS111906 for funding. We thank M. Müller for supplying the expression vector containing KtnC. We thank D. Sherman for supplying the expression vector containing RhFRed. We thank E. A. Meucci, E. C. Bornowski, and J. B. Pyser for assistance with the synthesis of substrates.

Competing Interests The authors have no conflicts of interest to report.

Supplementary information is available for this paper at xx.

Correspondence and requests for materials should be addressed to A.R.H.N.

References

- 1 Urabe, D., Asaba, T. & Inoue, M. Convergent strategies in total syntheses of complex terpenoids. *Chem. Rev.* **115**, 9207-9231 (2015).
- 2 Yin & Liebscher, J. Carbon–carbon coupling reactions catalyzed by heterogeneous palladium catalysts. *Chem. Rev.* **107**, 133-173 (2007).
- 3 Gaich, T. & Baran, P. S. Aiming for the ideal synthesis. *J. Org. Chem.* **75**, 4657-4673 (2010).
- 4 Pyser, J. B., Chakrabarty, S., Romero, E. O. & Narayan, A. R. H. State-of-the-art biocatalysis. *ACS Cent. Sci.* (2021).
- 5 Bornscheuer, U. T. *et al.* Engineering the third wave of biocatalysis. *Nature* **485**, 185-194 (2012).
- 6 Turner, N. J. & O'Reilly, E. Biocatalytic retrosynthesis. *Nat. Chem. Biol.* **9**, 285-288 (2013).
- 7 Prier, C. K. & Kosjek, B. Recent preparative applications of redox enzymes. *Curr. Opin. Chem. Biol.* **49**, 105-112 (2019).
- 8 Hauer, B. Embracing Nature's catalysts: a viewpoint on the future of biocatalysis. *ACS Catal.* **10**, 8418-8427 (2020).
- 9 Zetzsche, L. E. & Narayan, A. R. H. Broadening the scope of biocatalytic C–C bond formation. *Nat. Rev. Chem.* **4**, 334-346 (2020).
- 10 Chakrabarty, S., Romero, E. O., Pyser, J. B., Yazarians, J. A. & Narayan, A. R. H. Chemoenzymatic total synthesis of natural products. *Acc. Chem. Res.* **54**, 1374-1384 (2021).
- 11 Romero, E. *et al.* Enzymatic late-stage modifications: better late than never. *Angew. Chem. Int. Ed.* **60**, 2-34 (2021).
- 12 Chatterjee, A. *et al.* An enantioselective artificial Suzukiase based on the biotin–streptavidin technology. *Chem. Sci.* **7**, 673-677 (2016).
- 13 Schultz, E. E., Braffman, N. R., Luescher, M. U., Hager, H. H. & Balskus, E. P. Biocatalytic Friedel–Crafts alkylation using a promiscuous biosynthetic enzyme. *Angew. Chem. Int. Ed.* **58**, 3151-3155 (2019).
- 14 Huang, X. *et al.* Photoenzymatic enantioselective intermolecular radical hydroalkylation. *Nature* **584**, 69-74 (2020).
- 15 Page, C. G. *et al.* Quaternary charge-transfer complex enables photoenzymatic intermolecular hydroalkylation of olefins. *J. Am. Chem. Soc.* **143**, 97-102 (2021).
- 16 Yoon, T. P. & Jacobsen, E. N. Privileged chiral catalysts. *Science* **299**, 1691-1693 (2003).
- 17 Yet, L. in *Privileged Structures in Drug Discovery* Ch. 4, 83-154 (John Wiley & Sons, Inc., 2018).
- 18 Boström, J., Brown, D. G., Young, R. J. & Keserü, G. M. Expanding the medicinal chemistry synthetic toolbox. *Nat. Rev. Drug Discov.* **17**, 709-727 (2018).
- 19 Roughley, S. D. & Jordan, A. M. The medicinal chemist's toolbox: an analysis of reactions used in the pursuit of drug candidates. *J. Med. Chem.* **54**, 3451-3479 (2011).

- 20 Patel, N. D. *et al.* Computationally assisted mechanistic investigation and development of Pd-catalyzed asymmetric Suzuki–Miyaura and Negishi cross-coupling reactions for tetra-*ortho*-substituted biaryl synthesis. *ACS Catal.* **8**, 10190-10209 (2018).
- 21 Kozłowski, M. C. Oxidative coupling in complexity building transforms. *Acc. Chem. Res.* **50**, 638-643 (2017).
- 22 Yin, J., Rainka, M. P., Zhang, X.-X. & Buchwald, S. L. A highly active Suzuki catalyst for the synthesis of sterically hindered biaryls: novel ligand coordination. *J. Am. Chem. Soc.* **124**, 1162-1163 (2002).
- 23 Cammidge, A. N. & Crépy, K. V. L. Synthesis of chiral binaphthalenes using the asymmetric Suzuki reaction. *Tetrahedron* **60**, 4377-4386 (2004).
- 24 Organ, M. G., Çalimsiz, S., Sayah, M., Hoi, K. H. & Lough, A. J. Pd-PEPPSI-IPent: an active, sterically demanding cross-coupling catalyst and its application in the synthesis of tetra-*ortho*-substituted biaryls. *Angew. Chem. Int. Ed.* **48**, 2383-2387 (2009).
- 25 Valente, C. *et al.* The development of bulky palladium NHC complexes for the most-challenging cross-coupling reactions. *Angew. Chem. Int. Ed.* **51**, 3314-3332 (2012).
- 26 Wu, L. *et al.* Room-temperature synthesis of tetra-*ortho*-substituted biaryls by NHC-catalyzed Suzuki–Miyaura couplings. *Chem. Eur. J.* **17**, 12886-12890 (2011).
- 27 Ackermann, L., Potukuchi, H. K., Althammer, A., Born, R. & Mayer, P. Tetra-*ortho*-substituted biaryls through palladium-catalyzed Suzuki–Miyaura couplings with a diaminochlorophosphine ligand. *Org. Lett.* **12**, 1004-1007 (2010).
- 28 Brown, D. G., Gagnon, M. M. & Boström, J. Understanding our love affair with *p*-chlorophenyl: present day implications from historical biases of reagent selection. *J. Med. Chem.* **58**, 2390-2405 (2015).
- 29 Brown, D. G. & Boström, J. Analysis of past and present synthetic methodologies on medicinal chemistry: where have all the new reactions gone? *J. Med. Chem.* **59**, 4443-4458 (2016).
- 30 Grzybowski, M., Sadowski, B., Butenschön, H. & Gryko, D. T. Synthetic applications of oxidative aromatic coupling—from biphenols to nanographenes. *Angew. Chem. Int. Ed.* **59**, 2998-3027 (2020).
- 31 Yang, Y., Lan, J. & You, J. Oxidative C–H/C–H coupling reactions between two (hetero)arenes. *Chem. Rev.* **117**, 8787-8863 (2017).
- 32 Lee, Y. E., Cao, T., Torruellas, C. & Kozłowski, M. C. Selective oxidative homo- and cross-coupling of phenols with aerobic catalysts. *J. Am. Chem. Soc.* **136**, 6782-6785 (2014).
- 33 Nieves-Quinones, Y. *et al.* Chromium-salen catalyzed cross-coupling of phenols: mechanism and origin of the selectivity. *J. Am. Chem. Soc.* **141**, 10016-10032 (2019).
- 34 Shalit, H., Dyadyuk, A. & Pappo, D. Selective oxidative phenol coupling by iron catalysis. *J. Org. Chem.* **84**, 1677-1686 (2019).
- 35 Scott, A. I. Oxidative coupling of phenolic compounds. *Q. Rev. Chem. Soc.* **19**, 1-35 (1965).
- 36 McDonald, P. D. & Hamilton, G. A. in *Organic Chemistry* Vol. 5 (ed Walter S. Trahanovsky) 97-134 (Elsevier, 1973).
- 37 Libman, A. *et al.* Synthetic and predictive approach to unsymmetrical biphenols by iron-catalyzed chelated radical–anion oxidative coupling. *J. Am. Chem. Soc.* **137**, 11453-11460 (2015).
- 38 Shalit, H., Libman, A. & Pappo, D. *meso*-Tetraphenylporphyrin iron chloride catalyzed selective oxidative cross-coupling of phenols. *J. Am. Chem. Soc.* **139**, 13404-13413 (2017).
- 39 Wiebe, A., Riehl, B., Lips, S., Franke, R. & Waldvogel, S. R. Unexpected high robustness of electrochemical cross-coupling for a broad range of current density. *Sci. Adv.* **3**, eaao3920 (2017).

- 40 Kang, H. *et al.* Enantioselective vanadium-catalyzed oxidative coupling: development and mechanistic insights. *J. Org. Chem.* **83**, 14362-14384 (2018).
- 41 Yu, Q. *et al.* Enantioselective oxidative phenol-indole [3 + 2] coupling enabled by biomimetic Mn(III)/Brønsted acid relay catalysis. *ACS Catal.* **9**, 7285-7291 (2019).
- 42 Reiss, H. *et al.* Cobalt(II)[salen]-catalyzed selective aerobic oxidative cross-coupling between electron-rich phenols and 2-naphthols. *J. Org. Chem.* **84**, 7950-7960 (2019).
- 43 Sako, M. *et al.* Chiral dinuclear vanadium complex-mediated oxidative coupling of resorcinols. *J. Org. Chem.* **84**, 1580-1587 (2019).
- 44 Tian, J.-M. *et al.* Copper-complex-catalyzed asymmetric aerobic oxidative cross-coupling of 2-naphthols: enantioselective synthesis of 3,3'-substituted C1-symmetric BINOLs. *Angew. Chem. Int. Ed.* **58**, 11023-11027 (2019).
- 45 Xu, W., Huang, Z., Ji, X. & Lumb, J.-P. Catalytic aerobic cross-dehydrogenative coupling of phenols and catechols. *ACS Catal.* **9**, 3800-3810 (2019).
- 46 Hayashi, H., Ueno, T., Kim, C. & Uchida, T. Ruthenium-catalyzed cross-selective asymmetric oxidative coupling of arenols. *Org. Lett.* **22**, 1469-1474 (2020).
- 47 Neuhaus, W. C. & Kozlowski, M. C. Total synthesis of pyrolaside B: phenol trimerization through sequenced oxidative C–C and C–O coupling. *Angew. Chem. Int. Ed.* **59**, 7842-7847 (2020).
- 48 Paniak, T. J. & Kozlowski, M. C. Aerobic catalyzed oxidative cross-coupling of N,N-disubstituted anilines and aminonaphthalenes with phenols and naphthols. *Org. Lett.* **22**, 1765-1770 (2020).
- 49 Röckl, J. L., Schollmeyer, D., Franke, R. & Waldvogel, S. R. Dehydrogenative anodic C–C coupling of phenols bearing electron-withdrawing groups. *Angew. Chem. Int. Ed.* **59**, 315-319 (2020).
- 50 Wu, X. *et al.* Asymmetric synthesis of gonytolide A: strategic use of an aryl halide blocking group for oxidative coupling. *J. Am. Chem. Soc.* **140**, 5969-5975 (2018).
- 51 Bringmann, G., Gulder, T., Gulder, T. A. M. & Breuning, M. Atroposelective total synthesis of axially chiral biaryl natural products. *Chem. Rev.* **111**, 563-639 (2011).
- 52 Bringmann, G. *et al.* Atroposelective synthesis of axially chiral biaryl compounds. *Angew. Chem. Int. Ed.* **44**, 5384-5427 (2005).
- 53 Bringmann, G., Günther, C., Ochse, M., Schupp, O. & Tasler, S. in *Progress in the Chemistry of Organic Natural Products* (eds G. Bringmann *et al.*) 1-249 (Springer Vienna, 2001).
- 54 Aldemir, H., Richarz, R. & Gulder, T. A. The biocatalytic repertoire of natural biaryl formation. *Angew. Chem. Int. Ed.* **53**, 8286-8293 (2014).
- 55 Gil Girol, C. *et al.* Regio- and stereoselective oxidative phenol coupling in *Aspergillus niger*. *Angew. Chem. Int. Ed.* **51**, 9788-9791 (2012).
- 56 Präg, A. *et al.* Regio- and stereoselective intermolecular oxidative phenol coupling in *Streptomyces*. *J. Am. Chem. Soc.* **136**, 6195-6198 (2014).
- 57 Thiele, W., Froede, R., Steglich, W. & Müller, M. Enzymatic formation of rufoschweinitzin, a binaphthalene from the basidiomycete *Cortinarius rufoolivaceus*. *ChemBioChem* **21**, 1423-1427 (2020).
- 58 Mazzaferro, L. S., Huttel, W., Fries, A. & Muller, M. Cytochrome P450-catalyzed regio- and stereoselective phenol coupling of fungal natural products. *J. Am. Chem. Soc.* **137**, 12289-12295 (2015).
- 59 Obermaier, S. & Muller, M. Biaryl-forming enzymes from *Aspergilli* exhibit substrate-dependent stereoselectivity. *Biochemistry* **58**, 2589-2593 (2019).
- 60 Griffiths, S. *et al.* Elucidation of cladofulvin biosynthesis reveals a cytochrome P450 monooxygenase required for anthraquinone dimerization. *Proc. Natl. Acad. Sci.* **113**, 6851 (2016).

- 61 Matsuda, Y., Gotfredsen, C. H. & Larsen, T. O. Genetic characterization of neosartorin biosynthesis provides insight into heterodimeric natural product generation. *Org. Lett.* **20**, 7197-7200 (2018).
- 62 Funa, N., Funabashi, M., Ohnishi, Y. & Horinouchi, S. Biosynthesis of hexahydroxyperylenequinone melanin via oxidative aryl coupling by cytochrome P-450 in *Streptomyces griseus*. *J. Bacteriol.* **187**, 8149-8155 (2005).
- 63 Zhao, B. *et al.* Different binding modes of two flaviolin substrate molecules in cytochrome P450 158A1 (CYP158A1) compared to CYP158A2. *Biochemistry* **46**, 8725-8733 (2007).
- 64 Zhao, B. *et al.* Binding of two flaviolin substrate molecules, oxidative coupling, and crystal structure of *Streptomyces coelicolor* A3(2) cytochrome P450 158A2. *J. Biol. Chem.* **280**, 11599-11607 (2005).
- 65 Lim, Y.-R. *et al.* Characterization of a biflaviolin synthase CYP158A3 from *Streptomyces avermitilis* and its role in the biosynthesis of secondary metabolites. *Biomol. Ther.* **25**, 171-176 (2017).
- 66 Effenberger, I. *et al.* Dirigent proteins from cotton (*Gossypium* sp.) for the atropselective synthesis of gossypol. *Angew. Chem. Int. Ed.* **54**, 14660-14663 (2015).
- 67 Hu, J., Li, H. & Chooi, Y.-H. Fungal dirigent protein controls the stereoselectivity of multicopper oxidase-catalyzed phenol coupling in viriditoxin biosynthesis. *J. Am. Chem. Soc.* **141**, 8068-8072 (2019).
- 68 Obermaier, S., Thiele, W., Fürtges, L. & Müller, M. Enantioselective phenol coupling by laccases in the biosynthesis of fungal dimeric naphthopyrones. *Angew. Chem. Int. Ed.* **58**, 9125-9128 (2019).
- 69 Sagui, F. *et al.* Laccase-catalyzed coupling of catharanthine and vindoline: an efficient approach to the bisindole alkaloid anhydrovinblastine. *Tetrahedron* **65**, 312-317 (2009).
- 70 Ciecholewski, S. *et al.* Laccase-catalyzed carbon-carbon bond formation: oxidative dimerization of salicylic esters by air in aqueous solution. *Tetrahedron* **61**, 4615-4619 (2005).
- 71 Engelmann, C., Illner, S. & Kragl, U. Laccase initiated C–C couplings: Various techniques for reaction monitoring. *Process Biochem.* **50**, 1591-1599 (2015).
- 72 Zhang, X., Guo, J., Cheng, F. & Li, S. Cytochrome P450 enzymes in fungal natural product biosynthesis. *Nat. Prod. Rep.* **38**, 1072-1099 (2021).
- 73 Bischoff, D. *et al.* The biosynthesis of vancomycin-type glycopeptide antibiotics—the order of the cyclization steps. *Angew. Chem. Int. Ed.* **40**, 4688-4691 (2001).
- 74 Gesell, A. *et al.* CYP719B1 is salutaridine synthase, the C–C phenol-coupling enzyme of morphine biosynthesis in opium poppy. *J. Biol. Chem.* **284**, 24432-24442 (2009).
- 75 Cregg, J. M., Cereghino, J. L., Shi, J. & Higgins, D. R. Recombinant protein expression in *Pichia pastoris*. *Mol. Biotechnol.* **16**, 23-52 (2000).
- 76 Chakrabarty, S., Wang, Y., Perkins, J. C. & Narayan, A. R. H. Scalable biocatalytic C–H oxyfunctionalization reactions. *Chem. Soc. Rev.* **49**, 8137-8155 (2020).
- 77 Noji, M., Nakajima, M. & Koga, K. A new catalytic system for aerobic oxidative coupling of 2-naphthol derivatives by the use of CuCl-amine complex: a practical synthesis of binaphthol derivatives. *Tet. Lett.* **35**, 7983-7984 (1994).
- 78 Nakajima, M. Synthesis and application of novel biaryl compounds with axial chirality as catalysts in enantioselective reactions. *Yakugaku Zasshi* **120**, 68-75 (2000).
- 79 Feldman, K. S. & Ensel, S. M. Ellagitannin chemistry. Preparative and mechanistic studies of the biomimetic oxidative coupling of galloyl esters. *J. Am. Chem. Soc.* **116**, 3357-3366 (1994).

- 80 Langeslay, R. R. *et al.* Catalytic applications of vanadium: a mechanistic perspective. *Chem. Rev.* **119**, 2128-2191 (2018).
- 81 Hirao, T. Vanadium in modern organic synthesis. *Chem. Rev.* **97**, 2707-2724 (1997).
- 82 Kupchan, S. M., Dhingra, O. P. & Kim, C.-K. Efficient intramolecular monophenol oxidative coupling. *J. Org. Chem.* **43**, 4076-4081 (1978).
- 83 Shannon, P. *et al.* Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498-2504 (2003).
- 84 Gerlt, J. A. *et al.* Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST): a web tool for generating protein sequence similarity networks. *Biochim. Biophys. Acta.* **1854**, 1019-1037, doi:10.1016/j.bbapap.2015.04.015 (2015).
- 85 Zallot, R., Oberg, N. O. & Gerlt, J. A. 'Democratized' genomic enzymology web tools for functional assignment. *Curr. Opin. Chem. Biol.* **47**, 77-85 (2018).
- 86 Zallot, R., Oberg, N. & Gerlt, J. A. The EFI web resource for genomic enzymology tools: leveraging protein, genome, and metagenome databases to discover novel enzymes and metabolic pathways. *Biochemistry* **58**, 4169-4182 (2019).
- 87 Zhao, B. *et al.* Binding of two flavin substrate molecules, oxidative coupling, and crystal structure of *Streptomyces coelicolor* A3 (2) cytochrome P450 158A2. *J. Biol. Chem.* **280**, 11599-11607 (2005).
- 88 Zhao, B., Guengerich, F., Voehler, M. & Waterman, M. Role of active site water molecules and substrate hydroxyl groups in oxygen activation by cytochrome P450 158A2: a new mechanism of proton transfer. *J. Biol. Chem.* **280**, 42188-42197 (2006).
- 89 Li, S., Podust, L. M. & Sherman, D. H. Engineering and analysis of a self-sufficient biosynthetic cytochrome P450 PikC fused to the RhFRED reductase domain. *J. Am. Chem. Soc.* **129**, 12940-12941 (2007).
- 90 Brustad, E. M. & Arnold, F. H. Optimizing non-natural protein function with directed evolution. *Curr. Opin. Chem. Biol.* **15**, 201-210 (2011).
- 91 Chen, M. M. Y., Snow, C. D., Vizcarra, C. L., Mayo, S. L. & Arnold, F. H. Comparison of random mutagenesis and semi-rational designed libraries for improved cytochrome P450 BM3-catalyzed hydroxylation of small alkanes. *Protein Eng. Des. Sel.* **25**, 171-178 (2012).