# Enzymatic Primary Amination of C($sp^3$)–H Bonds

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**ABSTRACT:** Aliphatic primary amines are prevalent in natural products, pharmaceuticals, and functional materials. While a plethora of processes are reported for their synthesis, methods that directly install a primary amine group into unactivated C($sp^3$)–H bonds remain unprecedented. Here, we report a set of new-to-nature enzymes that catalyzes the direct primary amination of C($sp^3$)–H bonds with excellent chemo-, regio-, and enantioselectivity, using a readily available hydroxylamine derivative as the nitrogen source. Directed evolution of genetically-encoded cytochrome P411 enzymes (P450s whose Cys axial ligand to the heme iron has been replaced with Ser) generated variants that selectively functionalize benzylic and allylic C–H bonds, affording a broad scope of enantioenriched primary amines. This biocatalytic process is efficient and selective (up to 3930 TTN and 96% *ee*), and can be performed on preparative scale.

Nitrogen is a key component of functional molecules: 80% of small-molecule drugs, for example, contain at least one nitrogen atom.[1] Particularly privileged are primary amines that serve as essential intermediates in the construction of secondary amines, tertiary amines, and heterocycles.[2] A plethora of transformations have been developed to introduce the primary amine moiety into organic structures, given its importance as a fundamental functional group.[2,3] Classical synthesis of primary amines usually relies on manipulation of functional groups, such as reduction of azides or nitriles, reductive amination of carbonyl compounds,[4] and Buchwald-Hartwig amination of aryl halides.[5] Although C–H functionalization has emerged as a promising strategy that maximizes atom- and step-economy, state-of-the-art methods for amine synthesis typically provide *N*-protected or *N*-substituted products.[6] Recently, a few methods have been developed for primary amination of C($sp^2$)–H bonds, including photoredox catalysis,[7] electrochemical catalysis,[8] and the use of other novel amination reagents and metal catalysts.[9] Primary amination of C($sp^3$)–H bonds, however, has remained elusive.

Natural evolution has created diverse enzymes for selective C–H functionalization, ranging from hydroxylation to halogenation, under ambient conditions.[10] Among these, the direct hydroxylation of C($sp^3$)–H bonds catalyzed by cytochrome P450 enzymes (P450s) exemplifies the remarkable ability of enzymes to mediate transformations that are challenging for small-molecule catalysts. A high-valent iron oxo species, compound I, has been identified as the key intermediate.[11] The biocatalytic repertoire of P450s has been expanded through directed evolution to include the ability to generate and transfer abiotic nitrene and carbene intermediates.[12] We and Fasan, for example, engineered P450s to catalyze C–H amination via sulfonyl nitrene intermediates leading to the formation of diverse sulfonamides.[13] In spite of the prevalence and fundamental importance of aliphatic primary amines in the biological world, however, C($sp^3$)–H primary amination is unknown in biology.

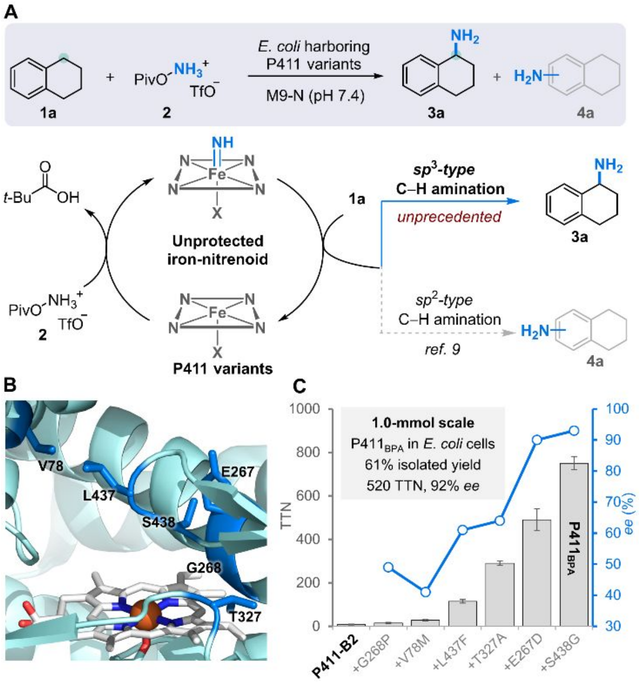


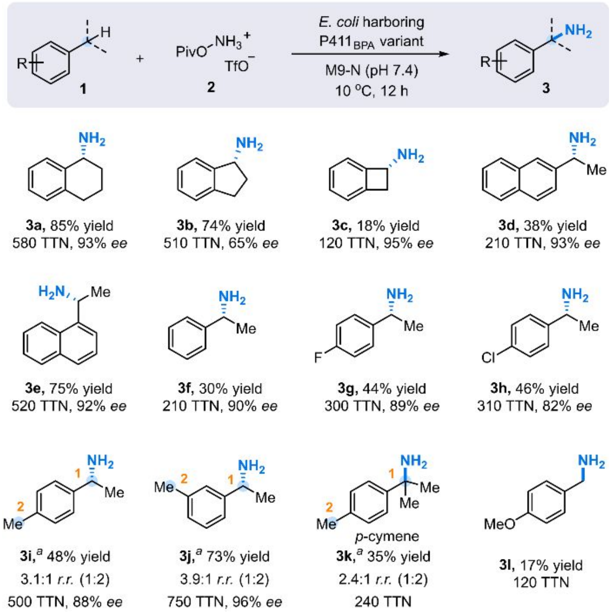
**Figure 1**. Enzymatic primary amination of benzylic C($sp^3$)–H bonds. (A) Reaction scheme of benzylic C($sp^3$)–H primary amination (Piv, pivaloyl). (B) Crystal structure of a variant (PDB ID: 5UCW) closely related to P411-B2, with mutated residues marked in blue.[13d] (C) Evolutionary trajectory of *benzylic C–H primary aminase* ($P411_{BPA}$) for the synthesis of benzylic amine **3a** and the scale-up reaction. $P411_{BPA}$ was evolved through six rounds of SSM and screening from P411-B2 (**Table S2**). Indicated mutations are relative to P411-B2. Unless otherwise noted, reaction conditions were as follows: P411 variants in *E. coli* whole cells [optical density at 600 nm ($OD_{600}$) = 16], 2.0 mM substrate **1a**, 4.0 mM substrate **2**, 2.5 vol% EtOH in M9-N (pH 7.4) buffer, 800 μL reaction volume at 10 ℃ under anaerobic conditions for 12 h. See the Supporting Information for further details of the scale-up reaction.

Here we addressed this challenge by directed evolution of a P450 from *Bacillus megaterium* ($P450_{BM3}$), which natively catalyzes C($sp^3$)–H hydroxylation of fatty acids. We hypothesized that a high-valent unprotected iron-nitrenoid intermediate would allow for direct C($sp^3$)–H primary amination, a process analogous to C–H hydroxylation through compound I. This iron-nitrenoid intermediate was demonstrated recently by us to enable aminohydroxylation of styrenyl olefins in an engineered cytochrome *c*.[14] Considering the highly tunable activities of $P450_{BM3}$ for C($sp^3$)–H functionalization conferred by the protein scaffold,[11a,12] we envisioned that directed evolution could divert and subsequently amplify the reactivity of the unprotected iron-nitrenoid species to primary amination of C($sp^3$)–H bonds. For this investigation we focused on engineered $P450_{BM3}$ variants that have the iron-coordinating cysteine residue replaced by serine (cytochrome 'P411s').[15]

We chose tetrahydronaphthalene **1a** as the model substrate to test in a reaction with the putative iron-nitrenoid generated from hydroxylamine ester **2** (**Figure 1A**). Although C($sp^2$)–H primary amination of substrate **1a** and its analogues is well established in synthetic chemistry,[9] primary amination of benzylic C($sp^3$)–H bonds remains unknown. Indeed, test reactions of **1a** and **2** with free heme only produced C($sp^2$)–H primary amination product **4a** (**Table S1**). The transformation was then evaluated with a panel of P411 variants derived from $P450_{BM3}$. The enzymes were expressed and screened in whole *Escherichia coli* cells, and the reactions were performed under anaerobic conditions in M9-N minimal medium. Gratifyingly, a variant named P411-B2, previously engineered for benzylic C–H amination via a sulfonyl nitrene,[13d] was identified to produce the desired primary benzylic amine **3a** with 8 total turnover number (TTN). Side product **4a** resulting from C($sp^2$)–H primary amination was also detected, in a negligible amount (< 1 TTN). These findings provided a starting point to test our hypothesis that protein engineering could divert and amplify the reactivity of the putative unprotected iron-nitrenoid to C($sp^3$)–H primary amination.

Variant P411-B2 was chosen as the starting template for directed evolution of an efficient *benzylic C–H primary aminase* ($P411_{BPA}$). Sequential rounds of site-saturation mutagenesis (SSM) and screening were performed to improve catalytic activity and enantioselectivity for the synthesis of benzylic amine **3a**. We referred to the crystal structure of a related P411 variant,[13d] and mainly chose amino acids proximal to the heme and/or residing on flexible loops for mutagenesis (**Figure 1B**). In each round of SSM, enzyme libraries were generated and screened by HPLC for product formation in 96-well plates in the form of whole-cell catalysts (see the Supporting Information for further detail). Four rounds of SSM and screening introduced mutations G268P, V78M, L437F, and T327A, leading to an increase of *ee* to 64% and a 36-fold improvement of TTN to 290 (**Figure 1C**). Subsequent rounds introduced mutations E267D and S438G, leading to final variant $P411_{BPA}$ and further boosting *ee* to 93% and TTN to 750 (**Table S2**). The formation of side product **4** remained negligible during directed evolution. Notably, the HPLC yield of **3a** in reactions at analytical scale was as high as 85%, and primary amine **3a** could be isolated in 61% yield and with 92% *ee* by using simple acid-base extraction (1.0 mmol reaction scale, see the Supporting Information for details).

With $P411_{BPA}$ in hand, we evaluated the substrate scope of alkanes, systematically targeting primary, secondary, and tertiary benzylic C–H bonds for primary C–H amination (**Figure 2**). For cyclic substrates bearing secondary benzylic C–H bonds, ring sizes ranging from four to six members were well tolerated, affording cyclic amines **3a–3c**. Linear substrates with structurally and electronically diverse aryl groups proved to be compatible and delivered amines **3d–3l** with excellent enantioselectivity. In addition to secondary benzylic C–H bonds (**3d–3j**), primary benzylic C–H bonds (**3i–3l**) and tertiary benzylic C–H bonds (**3k**) were also suitable with varying levels of efficiency and regioselectivity, highlighting the generality of this enzymatic transformation. Reaction at secondary and tertiary benzylic C–H bonds was more favorable than at their primary counterparts, as demonstrated by products **3i**, **3j**, and **3k**. Amination of larger substrates than those shown here or substrates with heteroatoms typically occurred with diminished efficiency, and further directed evolution based on $P411_{BPA}$ may be required for such substrates (**Figure S1**).



**3a**, 85% yield
580 TTN, 93% *ee*

**3b**, 74% yield
510 TTN, 65% *ee*

**3c**, 18% yield
120 TTN, 95% *ee*

**3d**, 38% yield
210 TTN, 93% *ee*

**3e**, 75% yield
520 TTN, 92% *ee*

**3f**, 30% yield
210 TTN, 90% *ee*

**3g**, 44% yield
300 TTN, 89% *ee*

**3h**, 46% yield
310 TTN, 82% *ee*

**3i**,[a] 48% yield
3.1:1 *r.r.* (1:2)
500 TTN, 88% *ee*

**3j**,[a] 73% yield
3.9:1 *r.r.* (1:2)
750 TTN, 96% *ee*

**3k**,[a] 35% yield
2.4:1 *r.r.* (1:2)
240 TTN

**3l**, 17% yield
120 TTN

**Figure 2.** Scope of benzylic amine products. Experiments were performed at analytical scale using enzyme-expressing *E. coli* cells resuspended to $OD_{600}$ = 16 or 24 in M9-N medium (pH 7.4), 2.0 mM substrate 1, 4.0 mM substrate 2, 2.5 vol% EtOH, and 800 µL reaction volume at 10 ℃ under anaerobic conditions for 12 hours. See **Table S3** for further detail. Regioisomeric ratio (*r.r.*) indicates the mole ratio of major product to combined minor regioisomers. [a] Yields and TTNs were calculated based on all regioisomers.

Having achieved primary amination of benzylic C–H bonds, we next asked whether the enzymes could aminate other types of C($sp^3$)–H bonds, particularly in the presence of sensitive olefins. With hydroxylamine derivatives as nitrogen sources and small-molecule catalysts, alkene substrates were reported to undergo olefin functionalization.[16] As expected, we observed only amino alcohol **7a** in the reaction of model substrate **5a** and hydroxylamine **2** with free heme as the catalyst (**Table S1**). Enzymes, however, often feature chemoselectivity in biochemical processes that can be tuned by protein engineering.[17] Therefore, we envisioned that appropriate engineering of $P411_{BPA}$-related enzymes would enable selective functionalization of allylic C–H bonds while minimizing the undesired olefin side reactions. To our delight, screening revealed that $P411_{BPA}$-related variants had the desired allylic C–H primary amination activity (**Figure 3A**). Byproduct **7a** was only formed in trace amounts (**Table S4**), and anilines resulting from C($sp^2$)–H primary amination were not observed. Starting from an intermediate variant $P411_{BPA'}$ having five mutations from P411-B2, we performed two rounds of SSM and screening to generate *allylic C–H primary aminase* ($P411_{APA}$) with two additional mutations (N395R, S438A). $P411_{APA}$ is exceptionally efficient and selective (3930 TTN, 93% HPLC yield, and 94% *ee*), and the enzymatic process is easily scalable, with 75% isolated yield (1.0 mmol reaction scale, see the Supporting Information for details).

$P411_{APA}$ is capable of functionalizing primary, secondary, and tertiary allylic C–H bonds with a broad substrate scope (**Figure 3B**). For styrene-type substrates bearing secondary allylic C–H bonds, phenyl groups with varying substituents were tolerated well to afford allylic amines **6a–6g** with excellent activity and enantioselectivity. In the presence of primary benzylic C–H bonds, allylic C–H bonds were functionalized with complete regioselectivity (**6e–6g**). Substrates with heterocyclic substituents such as thiophenyl (**6h**) and longer aliphatic substituents (**6i**) also proved to be suitable. Single regioisomer **6j** was exclusively afforded, indicating $P411_{APA}$ can discriminate between highly similar secondary allylic C–H bonds. Tertiary and primary allylic C–H bonds could also be aminated efficiently to give products **6k** and **6l**. Notably, aromatic groups on the alkene moiety are not necessary for allylic C–H amination activity, and products **6m–6p** were successfully synthesized. For substrates with different types of reactive C–H bonds, P411$_{APA}$ exhibits high levels of regioselectivity. While 2-hexene gave a mixture of regioisomers (**6n**), single regioisomers were observed for other alkenes with up to four sets of allylic C–H bonds (**3o**, **3p**), such as α-terpinene. Although the regioselectivity between secondary and primary C–H bonds is dependent on substrate patterns (**6n**, **6p**), P411$_{APA}$ exclusively functionalized primary C–H bonds over weaker tertiary C–H bonds (**6o**, **6p**), which is contrary to the regioselectivity of P411$_{BPA}$ (**3k**). Similar to P411$_{BPA}$, further directed evolution of P411$_{APA}$ may be required for efficient functionalization of terminal or larger alkene substrates, especially complex natural products (**Figure S1**).

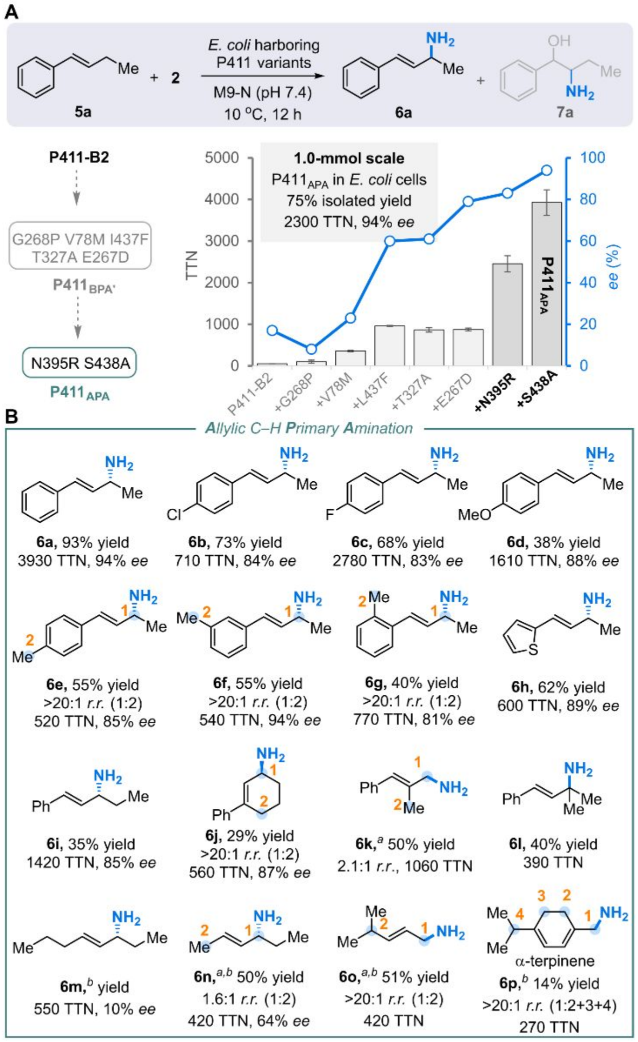


**Figure 3.** Engineering enzymes for allylic C–H primary amination and the substrate scope. (A) Evolutionary trajectory of *allylic C–H primary aminase* (P411$_{APA}$) for the synthesis of allylic amine **6a** and the scale-up reaction. P411$_{APA}$ was evolved through two more rounds of SSM and screening starting from an intermediate benzylic C–H aminase having five mutations with respect to P411-B2 (**Table S4**). The indicated mutations are relative to P411-B2. (B) Substrate scope of allylic C–H primary amination. See **Table S5** for more details. $^{a}$ Yields and TTNs were calculated based on all regioisomers. $^{b}$ Products were characterized and quantified by their benzoyl-protected amines.

In conclusion, we have engineered *C(sp$^3$)–H primary aminases* that constitute a general platform for selective primary amination of primary, secondary, and tertiary C–H bonds at benzylic and allylic positions. These laboratory-evolved enzymes provide a new approach to accessing aliphatic primary amines with high chemoselectivity, regioselectivity, and enantioselectivity. Considering the fundamental roles of primary amines in the biological world and the natural occurrence and biocompatibility of hydroxylamine derivatives, we anticipate that these new, fully genetically-encoded enzymes will provide a starting point to extend or even reformulate currently mapped nitrogen metabolism. Such activities may, in fact, already exist in nature, whose vast catalytic capabilities have only been partially explored. Further elucidation of the catalytic mechanism and characterization of the key intermediate would be valuable to generalize this process and inspire the design of corresponding small-molecule catalysts.

## ASSOCIATED CONTENT

### Supporting Information

Materials, experimental methods, and compound characterization data, including Tables S1−S6 and Figure S1.

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### Notes

Caltech has filed a provisional patent application.

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## REFERENCES

(1) (a) Hili, R.; Yudin, A. K. Making Carbon-Nitrogen Bonds in Biological and Chemical Synthesis. *Nat. Chem. Biol*. **2006**, *2*, 284–287. (b) Vitaku, E.; Smith, D. T.; Njardarson, J. T. Analysis of the Structural Diversity, Substitution Patterns, and Frequency of Nitrogen Heterocycles among U.S. FDA Approved Pharmaceuticals. *J. Med. Chem.* **2014**, *57*, 10257–10274.
(2) *Amino Group Chemistry: From Synthesis to the Life Sciences*; Ricci, A., Ed.; Wiley-VCH: Weinheim, Germany, **2008**.
(3) Legnani, L.; Bhawal, B. N.; Morandi, B. Recent Developments in the Direct Synthesis of Unprotected Primary Amines. *Synthesis* **2017**, *49*, 776–789.
(4) Gross, T.; Seayad, A. M.; Ahmad, M.; Beller, M. Synthesis of Primary Amines: First Homogeneously Catalyzed Reductive Amination with Ammonia. *Org. Lett*. **2002**, *4*, 2055–2058.
(5) (a) Hartwig, J. F. Evolution of a Fourth Generation Catalyst for the Amination and Thioetherification of Aryl Halides. *Acc. Chem. Res*. **2008**, *41*, 1534–1544. (b) Surry, D. S.; Buchwald, S. L. Biaryl

Phosphane Ligands in Palladium-Catalyzed Amination. *Angew. Chem, Int. Ed.* **2008**, *47*, 6338–6361.

(6) Park, Y.; Kim, Y.; Chang, S. Transition Metal-Catalyzed C–H Amination: Scope, Mechanism, and Applications. *Chem. Rev*. **2017**， *117*, 9247–9301.

(7) (a) Romero, N. A.; Margrey, K. A.; Tay, N. E.; Nicewicz, D. A. Site-Selective Arene C–H Amination via Photoredox Catalysis. *Science* **2015**, *349*, 1326–1330. (b) Zheng, Y.-W.; Chen, B.; Ye, P.; Feng, K.; Wang, W.; Meng, Q.-Y.; Wu, L.-Z.; Tung, C.-H. Photocatalytic Hydrogen-Evolution Cross-Couplings: Benzene C–H Amination and Hydroxylation. *J. Am. Chem. Soc.* **2016**, *138*, 10080–10083.

(8) Morofuji, T.; Shimizu, A.; Yoshida, J.-I. Electrochemical C–H Amination: Synthesis of Aromatic Primary Amines via *N*-Arylpyridinium Ions. *J. Am. Chem. Soc*. **2013**, *135*, 5000–5003.

(9) (a) Peng, J.; Chen, M.; Xie, Z.; Luo, S.; Zhu, Q. Copper-Mediated C(sp$^2$)–H Amination Using TMSN$_3$ as a Nitrogen Source: Redox-Neutral Access to Primary Anilines. *Org. Chem. Front*. **2014**, *1*, 777–781. (b) Tezuka, N.; Shimojo, K.; Hirano, K.; Komagawa, S.; Yoshida, K.; Wang, C.; Miyamoto, K.; Saito, T.; Takita, R.; Uchiyama, M. Direct Hydroxylation and Amination of Arenes via Deprotonative Cupration. *J. Am. Chem. Soc*. **2016**, *138*, 9166–9171. (c) Paudyal, M. P.; Adebesin, A. M.; Burt, S. R.; Ess, D. H.; Ma, Z.; Kürti, L.; Falck, J. R. Dirhodium-Catalyzed C–H Arene Amination Using Hydroxylamines. *Science* **2016**, *353*, 1144–1147. (d) Legnani, L.; Prina Cerai, G.; Morandi, B. Direct and Practical Synthesis of Primary Anilines through Iron-Catalyzed C–H Bond Amination. *ACS Catal*. **2016**, *6*, 8162–8165. (e) Liu, J.; Wu, K.; Shen, T.; Liang, Y.; Zou, M.; Zhu, Y.; Li, X.; Li, X.; Jiao, N. Fe-Catalyzed Amination of (Hetero)Arenes with a Redox-Active Aminating Reagent under Mild Conditions. *Chem. Eur. J.* **2017**, *23*, 563–567. (f) Kim, H.; Heo, J.; Kim, J.; Baik, M.-H.; Chang, S. Copper-Mediated Amination of Aryl C–H Bonds with the Direct Use of Aqueous Ammonia via a Disproportionation Pathway. *J. Am. Chem. Soc.* **2018**, *140*, 14350–14356. (*g*) D'Amato, E. M.; Börgel, J.; Ritter, T. Aromatic C–H Amination in Hexafluoroisopropanol. *Chem. Sci*. **2019**, *10*, 2424–2428. (h) Anugu, R. R.; Munnuri, S.; Falck, J. R. Picolinate-Directed Arene *meta*-C–H Amination via FeCl$_3$ Catalysis. *J. Am. Chem. Soc*. **2020**, *142*, 5266–5271.

(10) Lewis, J. C.; Coelho, P. S.; Arnold, F. H. Enzymatic Functionalization of Carbon–Hydrogen Bonds. *Chem. Soc. Rev.* **2011**, *40*, 2003–2021.

(11) (a) Ortiz de Montellano, P. R. Hydrocarbon Hydroxylation by Cytochrome P450 Enzymes. *Chem. Rev*. **2010**, *110*, 932–948. (b) Rittle, J.; Green, M. T. Cytochrome P450 Compound I: Capture, Characterization, and C-H Bond Activation Kinetics. *Science* **2010**, *330*, 933–937.

(12) (a) Brandenberg, O. F.; Fasan, R.; Arnold, F. H. Exploiting and Engineering Hemoproteins for Abiological Carbene and Nitrene Transfer Reactions. *Curr. Opin. Biotechnol*. **2017**, *47*, 102–111. (b) Fasan, R.; Kan, S. B.; Zhao, H. A Continuing Career in Biocatalysis: Frances H. Arnold. *ACS Catal*. **2019**, *9*, 9775–9788. (c) Chen, K.; Arnold, F. H. Engineering New Catalytic Activities in Enzymes. *Nat. Catal.* **2020**, *3*, 203–213. (d) Singh, R.; Kolev, J. N.; Sutera, P. A.; Fasan, R. Enzymatic C(sp$^3$)-H Amination: P450-Catalyzed Conversion of Carbonazidates into Oxazolidinones. *ACS Catalysis* **2015**, *5*, 1685–1691. (e) Key, H. M.; Dydio, P.; Clark, D. S.; Hartwig, J. F. Abiological Catalysis by Artificial Haem Proteins Containing Noble Metals in Place of Iron. *Nature* **2016**, *534*, 534–537. (f) Dydio, P.; Key, H. M.; Hayashi, H.; Clark, D. S.; Hartwig, J. F. Chemoselective, Enzymatic C–H Bond Amination Catalyzed by a Cytochrome P450 Containing an Ir(Me)-PIX Cofactor. *J. Am. Chem. Soc*. **2017**, *139*, 1750–1753.

(13) (a) McIntosh, J. A.; Coelho, P. S.; Farwell, C. C.; Wang, Z. J.; Lewis, J. C.; Brown, T. R.; Arnold, F. H. Enantioselective Intramolecular C–H Amination Catalyzed by Engineered Cytochrome P450 Enzymes in vitro and in vivo. *Angew. Chem, Int. Ed.* **2013**, *52*, 9309–9312. (b) Hyster, T. K.; Farwell, C. C.; Buller, A. R.; McIntosh, J. A.; Arnold, F. H. Enzyme-Controlled Nitrogen-Atom Transfer Enables Regiodivergent C–H Amination. *J. Am. Chem. Soc.* **2014**, *136*, 15505–15508. (c) Singh, R.; Bordeaux, M.; Fasan, R. P450-Catalyzed Intramolecular sp$^3$ C–H Amination with Arylsulfonyl Azide Substrates. *ACS Catalysis* **2014**, *4*, 546–552. (d) Prier, C. K.; Zhang, R. K.; Buller, A. R.; Brinkmann-Chen, S.; Arnold, F. H. Enantioselective, Intermolecular Benzylic C–H Amination Catalysed by an Engineered Iron-Haem Enzyme. *Nat. Chem*. **2017**, *9*, 629–634. (e) Yang, Y.; Cho, I.; Qi, X.; Liu, P.; Arnold, F. H. An Enzymatic Platform for the Asymmetric Amination of Primary, Secondary and Tertiary C(*sp*$^3$)–H Bonds. *Nat. Chem*. **2019**, *11*, 987–993.

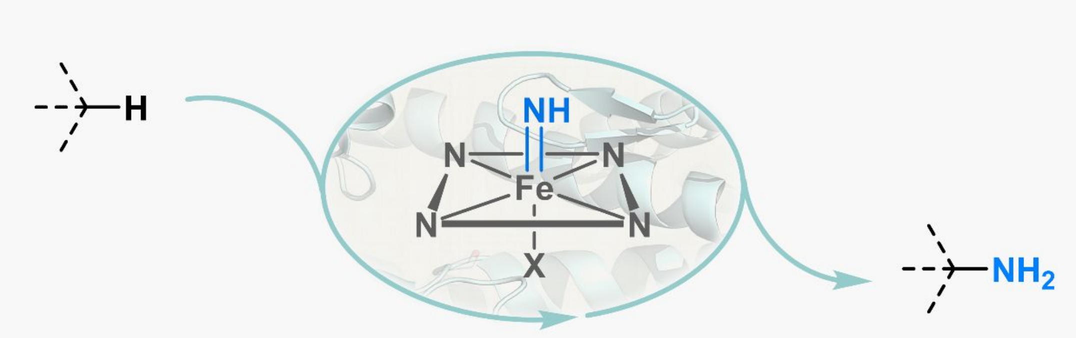
(14) Cho, I.; Prier, C. K.; Jia, Z.-J.; Zhang, R. K.; Görbe, T.; Arnold, F. H. Enantioselective Aminohydroxylation of Styrenyl Olefins Catalyzed by an Engineered Hemoprotein. *Angew. Chem, Int. Ed.* **2019**, *58*, 3138–3142.

(15) Coelho, P. S.; Wang, Z. J.; Ener, M. E.; Baril, S. A.; Kannan, A.; Arnold, F. H.; Brustad, E. M. A Serine-Substituted P450 Catalyzes Highly Efficient Carbene Transfer to Olefins i*n vivo*. *Nat. Chem. Biol.* **2013**, *9*, 485–487.

(16) (a) Jat, J. L.; Paudyal, M. P.; Gao, H. Y.; Xu, Q. L.; Yousufuddin, M.; Devarajan, D.; Ess, D. H.; Kurti, L.; Falck, J. R. Direct Stereospecific Synthesis of Unprotected N-H and N-Me Aziridines from Olefins. *Science* **2014**, *343*, 61–65. (b) Legnani, L.; Morandi, B. Direct Catalytic Synthesis of Unprotected 2-Amino-1-Phenylethanols from Alkenes by Using Iron(II) Phthalocyanine. *Angew. Chem. Int. Ed*. **2016**, *55*, 2248–2251. (c) Ma, Z.; Zhou, Z.; Kürti, L. Direct and Stereospecific Synthesis of *N*-H and *N*-Alkyl Aziridines from Unactivated Olefins Using Hydroxylamine-*O*-Sulfonic Acids. *Angew. Chem, Int. Ed*. **2017**, *56*, 9886–9890. (d) Legnani, L.; Prina-Cerai, G.; Delcaillau, T.; Willems, S.; Morandi, B. Efficient Access to Unprotected Primary Amines by Iron-Catalyzed Aminochlorination of Alkenes. *Science* **2018**, *362*, 434–439. (e) Zhou, Z.; Cheng, Q.-Q.; Kürti, L. Aza-Rubottom Oxidation: Synthetic Access to Primary α-Aminoketones. *J. Am. Chem. Soc*. **2019**, *141*, 2242–2246. (f) Cheng, Q.-Q.; Zhou, Z.; Jiang, H.; Siitonen, J. H.; Ess, D. H.; Zhang, X.; Kürti, L. Organocatalytic Nitrogen Transfer to Unactivated Olefins via Transient Oxaziridines. *Nat. Catal*. **2020**, DOI: 10.1038/s41929-020-0430-4.

(17) Benkovic, S. J.; Hammes-Schiffer, S. A Perspective on Enzyme Catalysis. *Science* **2003**, *301*, 1196–1202.

SYNOPSIS TOC



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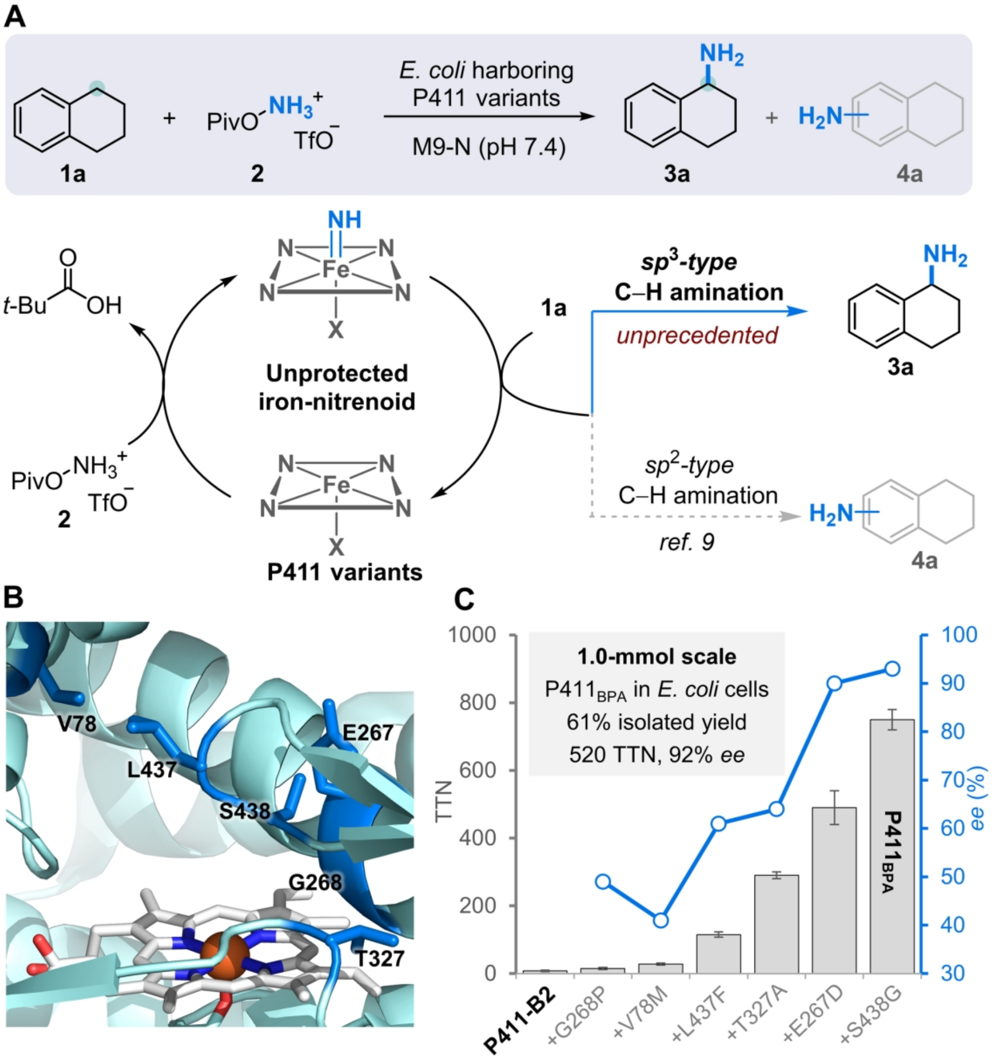

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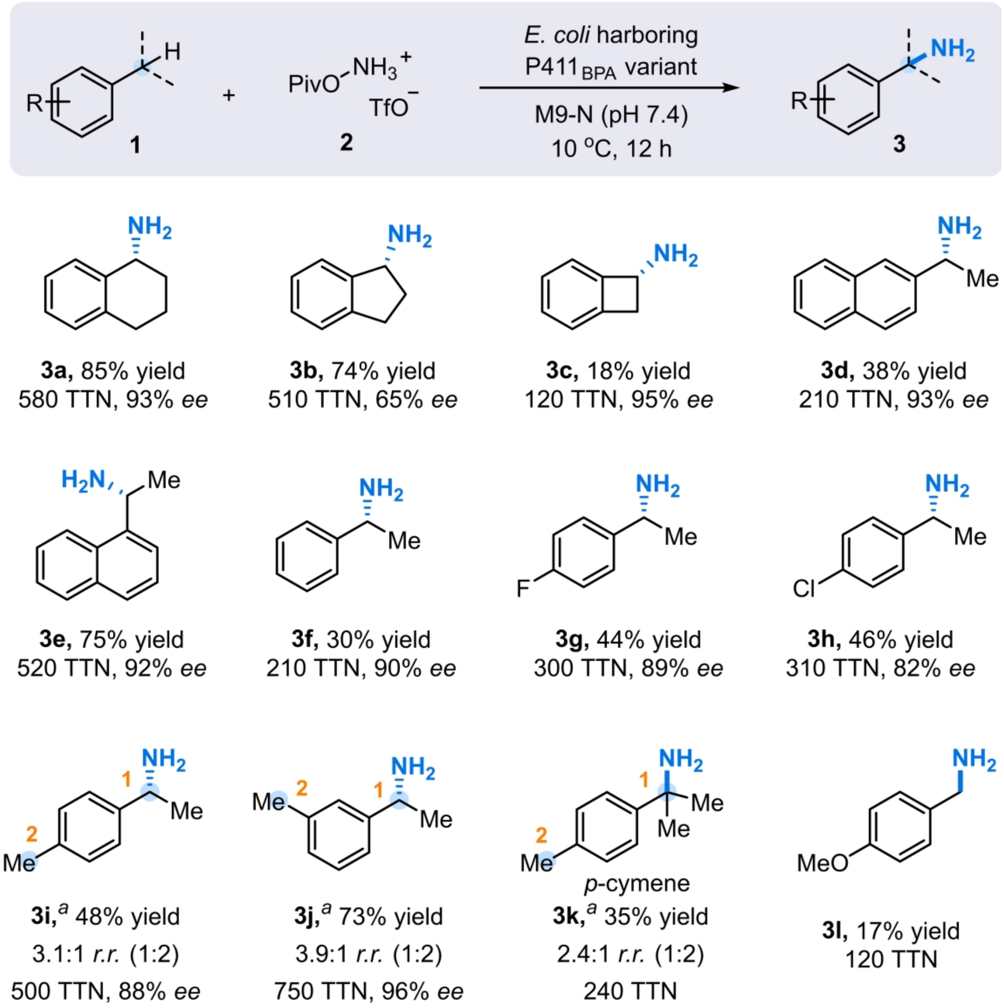

**Figure 2.** Scope of benzylic amine products. Experiments were performed at analytical scale using enzyme-expressing *E. coli* cells resuspended to $OD_{600}$ = 16 or 24 in M9-N medium (pH 7.4), 2.0 mM substrate 1, 4.0 mM substrate 2, 2.5 vol% EtOH, and 800 μL reaction volume at 10 ℃ under anaerobic conditions for 12 hours. See **Table S3** for further details. Regioisomeric ratio (*r.r.*) indicates the mole ratio of major product to combined minor regioisomers. [a] Yields and TTNs were calculated based on all regioisomers.

145x147mm (600 x 600 DPI)

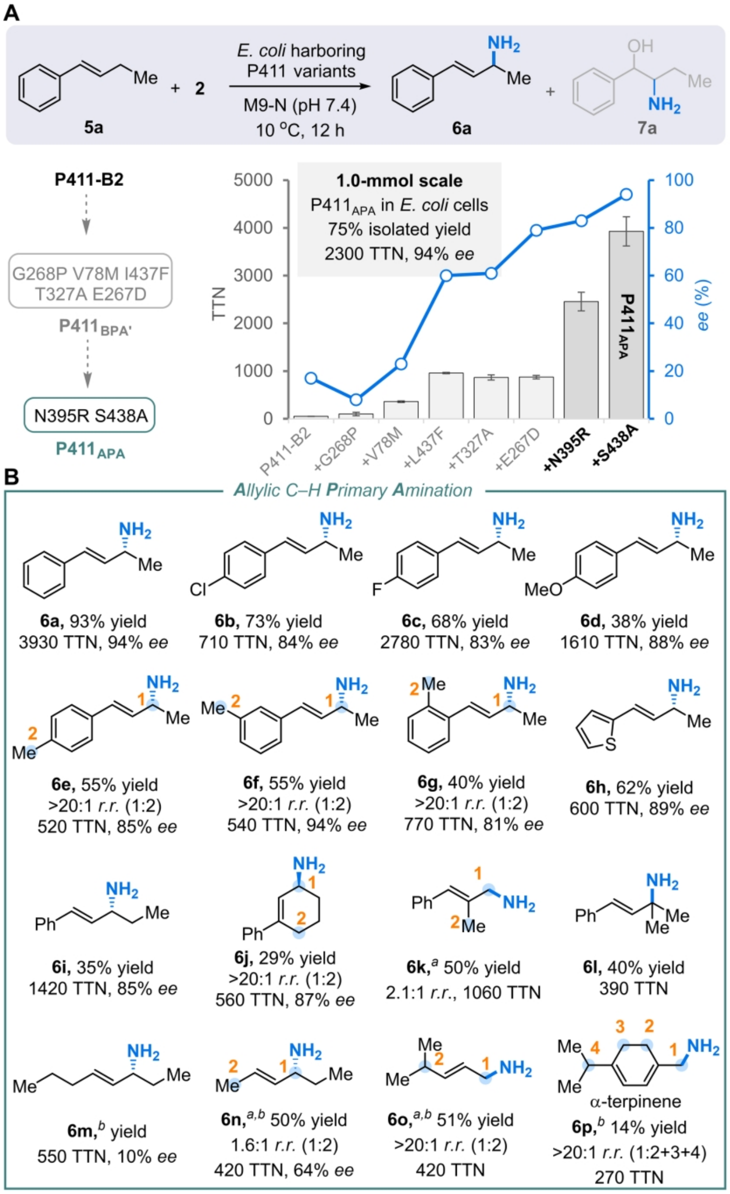

**Figure 3.** Engineering enzymes for allylic C–H primary amination and the substrate scope. (A) Evolutionary trajectory of *allylic C–H primary aminase* ($P411_{APA}$) for the synthesis of allylic amine **6a** and the scale-up reaction. $P411_{APA}$ was evolved through two more rounds of SSM and screening based on an intermediate benzylic C–H aminase having five mutations with respect to P411-B2 (**Table S4**). The indicated mutations are relative to P411-B2. (B) Substrate scope of allylic C–H primary amination. See **Table S5** for more details. $^{a}$ Yields and TTNs were calculated based on all regioiso-mers. $^{b}$ Products were characterized and quantified by their benzoyl-protected amines.

160x261mm (300 x 300 DPI)