Site-Selective Enzymatic C–H Amidation for Synthesis of Diverse Lactams

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A paramount challenge in carbon–hydrogen (C–H) functionalization is to control the site selectivity of the reaction. Current methods use directing groups and/or substrate control to pick out a particular C–H bond, which limits the breadth of potential substrates. Recently, an enzymatic strategy to address this challenge was reported by Professor Frances H. Arnold and coworkers Inha Cho (PhD student) and Dr. Zhi-Jun Jia (postdoctoral fellow) from the California Institute of Technology (USA). The authors used directed evolution to tune the site selectivity of C–H amidation catalyzed by heme enzymes. Professor Arnold explained: "Enzymes offer unparalleled selectivity in an array of transformations devised first by chemists and now established in natural metalloproteins. It's a splendid opportunity to merge human chemical ingenuity with the power of evolution to make new, synthetically useful catalysts."

The Arnold group's study uses an iron-heme cytochrome 'P411' to perform a C–H amidation transformation not found in nature. With directed evolution, the researchers fine-tuned the site selectivity of intramolecular amidation to deliver lactam products of various sizes (Figure 1A). Four different enzyme variants, LS_{sp3}, LS_{sp2}, LS_{β} and LS_{γ}, were evolved from a single parent to target specific C–H bonds at selected sites. "Notably, the enzymes can override reactivity trends due to C–H bond strength, inductive effects, steric accessibility and/or ring strain to deliver desired lactam products with broad substrate scope, excellent regioselectivity and enantioselectivity, and as many as one million total turnovers (TTN)," said Professor Arnold. Starting from a parent enzyme with low activity, six amino acid mutations in the enzyme's active site boosted the TTN by more than 500-fold for β-lactam synthesis (Figure 1B). This transformation can be performed on preparative scale and some products can be recovered easily by filtration from the reaction mixture (Figure 1C).

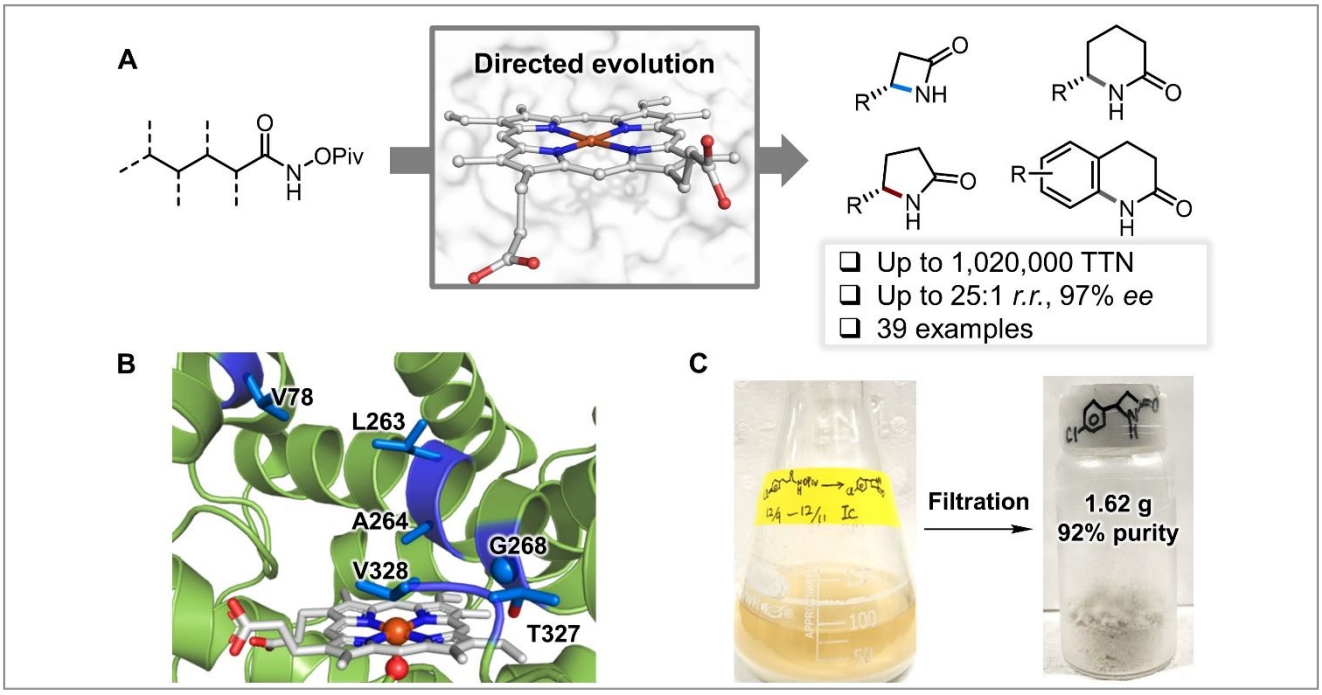


Figure 1 (A) General reaction scheme. (B) Crystal structure of a related variant (PDB ID: 5UCW), with mutated residues marked in blue (for LS_{sp3}). (C) Gram-scale synthesis.

The enzymes use carbonyl nitrenes – previously thought ineffective for C–H amidation due to their instability – for this reaction. The inspiration for evolving heme proteins to utilize carbonyl nitrenes came from earlier work. PhD student Inha Cho explained: "Previously we used *O*-pivaloylhydroxylamine as nitrene precursors for hemoprotein-catalyzed aminohydroxylation of olefins. The pivaloyl leaving group was highly effective for enhancing the yield and enantioselectivity of enzyme-catalyzed nitrene transfer. In 2017, Tsutsumi and co-workers (see the original paper for references) reported an intramolecular aziridination catalyzed by wild-type cytochrome P450s that use acyl-protected hydroxylamines as natural nitrene precursors in natural product biosynthesis. We reasoned that our P411s might accommodate acyl-protected hydroxamates to generate nitrene intermediates for C–H amidation." Upon screening a collection of more than 200 enzymes, Cho found an engineered variant of $P450_{BM3}$ that made a small amount of lactam product from a pivaloyl-protected hydroxamate precursor. She used this enzyme as a starting point for evolution of a 'lactam synthase'.

"During mutagenesis and screening, we found some enzymes that delivered mixtures of different lactams from the same substrate, presumably due to non-optimal control of regioselectivity. We believed that these enzymes could be engineered to form lactams of specific sizes, exclusively," explained Dr. Zhi-Jun Jia. They eventually evolved four lactam synthases, LS_{sp3}, LS_{sp2}, LSβ and LSγ, which target different C–H bonds in the same substrate. LS_{sp3} and LS_{sp2} catalyze C(sp^3)–H amidation and C(sp^2)–H amidation, respectively, and enable efficient and selective β-lactam and δ-lactam synthesis (Figure 2A). The reactivity and selectivity profiles of LS_β and LS_γ on substrates with benzylic and homobenzylic C–H bonds (Figure 2B) demonstrate the tunability of enzyme-catalyzed C–H amidation. Finally, from a substrate with three sets of reactive C(sp^3)–H bonds (Figure 2C), LS_β, LS_γ and LS_{sp3} afforded β-, γ- and δ-lactams, respectively.

The 'lactam synthases' were used to prepare a range of β-lactams (Figure 3). "Their unique structural features and *in vivo* reactivity make β-lactams versatile chemical building blocks as well as medicinal agents with antibacterial activity.

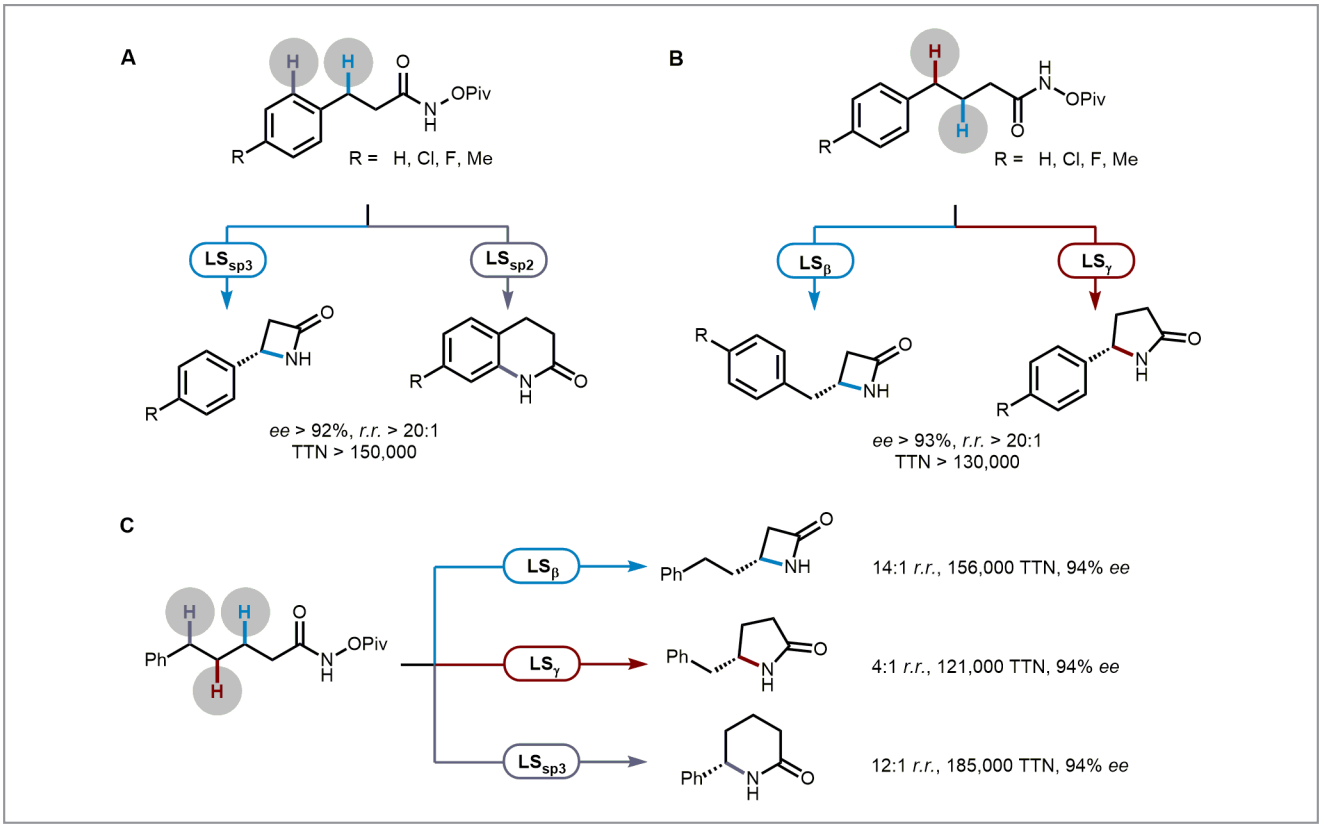


Figure 2 (A) Selectivity and scope of LS_{sp2} and LS_{sp3}-catalyzed intramolecular C–H amidation. (B) Selectivity and scope of LS_β and LS_γ. (C) Regiodivergent amidation of C(sp^3)–H bonds catalyzed by LS_β, LS_γ, and LS_{sp3}.

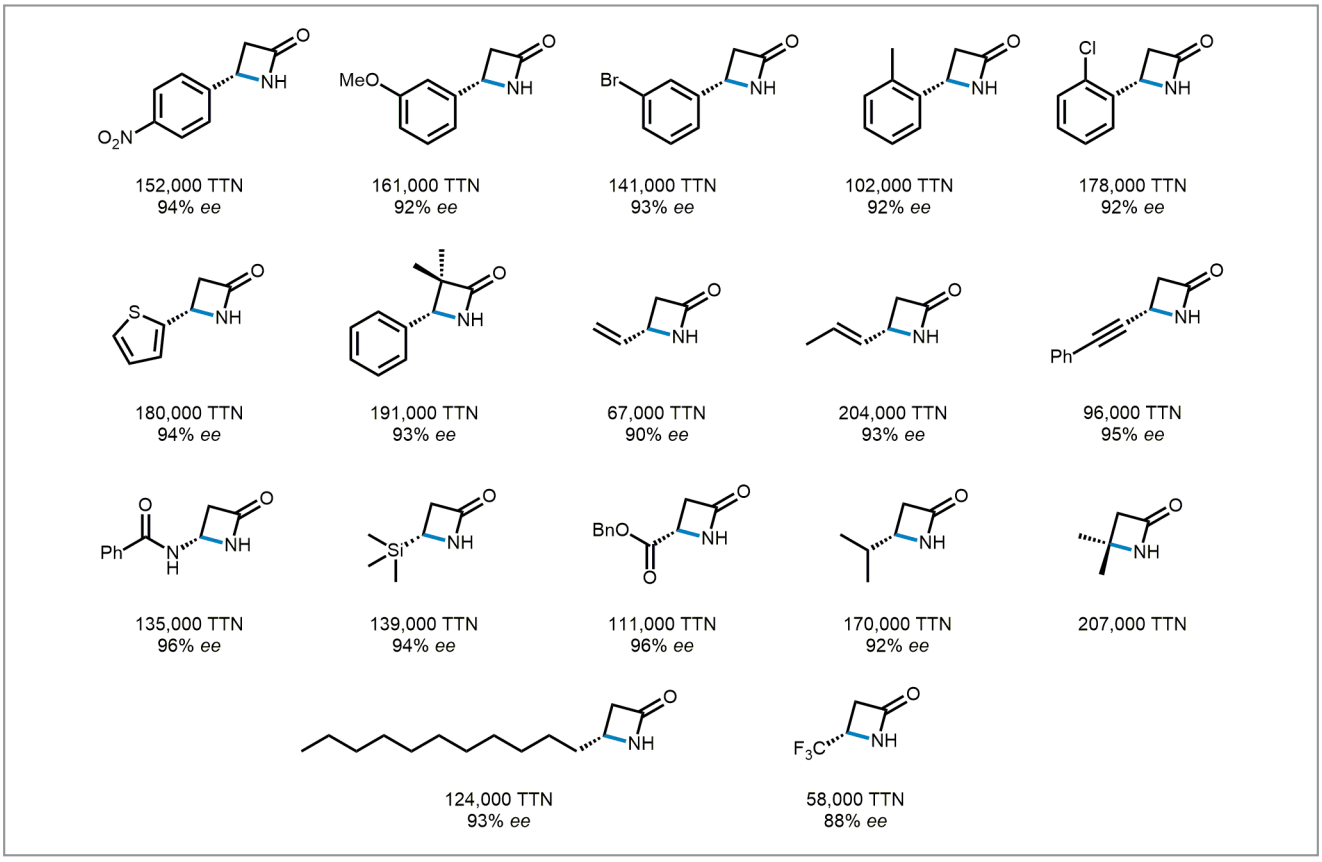


Figure 3 Range of β-lactam products synthesized with this method.

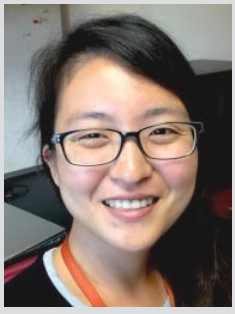
Since the discovery of penicillin in 1929, extensive use of antibiotics has led to the emergence of antibiotic resistance and significantly limited future therapeutic options," explained the authors of this study. "To feed new β-lactam antibiotics to the pipeline, a general synthetic platform is desirable. We hope that this work could provide a modular, sustainable and scalable method to efficiently construct diverse libraries of chiral β-lactams for drug development. Additionally, the enzymes are easy to prepare in *E. coli*, and the enzymatic reaction is robust." They concluded: "We hope that chemists will be able to use enzymes for synthesis in the same way we use small-molecule catalysts today."

Professor David O'Hagan from St. Andrews University (UK), who is an expert on the use of enzymes in organic synthesis, commented: "This report in *Science* is particularly exciting as the enzyme is induced to generate nitrenes, intermediates we rarely associate with enzymology due to their high reactivity, and then various reaction modes are channeled by evolving and selecting for different outcomes. Direct methods to form C–N bonds have had a major impact in modern organic and medicinal chemistry, so engineering this group of biocatalysts is particularly impressive and raises the bar. It shows that protein engineering has the power to harness catalysts for any reaction we are prepared to focus on, even those we would not normally associate with enzymes."

About the authors

Prof. F. H. Arnold

Frances H. Arnold earned a Bachelor's degree in mechanical and aerospace engineering from Princeton University (USA) and a doctorate in chemical engineering from the University of California at Berkeley (USA). She is the Linus Pauling Professor of Chemical Engineering, Bioengineering, and Biochemistry at the California Institute of Technology (USA). In 2018, she was awarded the Nobel Prize in Chemistry for her work on directed evolution of enzymes.



I. Cho

Inha Cho is from Seoul, Korea. She studied at Pohang University of Science and Technology (Korea) for a semester and moved to the USA to receive her B.A. in physics from Wesleyan University (CT, USA) under the guidance of Dr. Christina M. Othon, who is now an associate professor of physics at Ripon College. Inha Cho then joined Dr. Frances H. Arnold's group at the California Institute of Technology (USA) as a graduate student in biochemistry and molecular biophysics. Her main research focus is to engineer iron hemoproteins for abiological nitrene transfer chemistry.

Dr. Z.-J. Jia

Zhi-Jun Jia was born in Sichuan, P. R. of China. He received his B.S. in biology as well as marketing from Sichuan University (P. R. of China), and his M.S. degree in medicinal chemistry at the same university in 2012 under the guidance of Prof. Ying-Chun Chen. In 2016, he earned his Ph.D. in chemical biology under the supervision of Prof. Dr. Herbert Waldmann at the Max-Planck Institute for Molecular Physiology (Germany), working in biology-oriented synthesis. After one year of postdoctoral research in the same group, he joined Prof. Dr. Frances H. Arnold's lab as a DFG (Deutsche Forschungsgemeinschaft) postdoctoral fellow in 2018, where he is repurposing natural metalloenzymes for abiotic transformations through directed evolution.

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