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Biocatalytic activation of diazirines for carbene-transfer reactions

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Diazirines have been traditionally associated with chemical biology and materials chemistry, but their utility has been limited in organic chemistry. Recently in the *Journal of the American Chemical Society*, Arnold and co-workers employed diazirines as precursors to hemoprotein-derived metal carbenoid species.

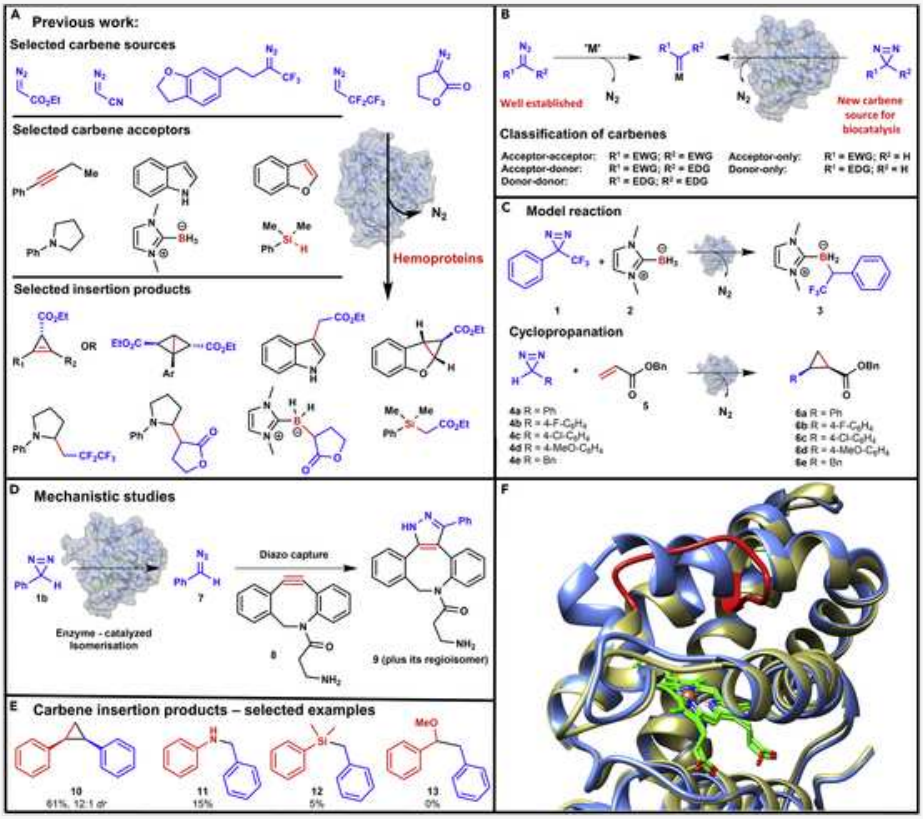
Q2Q3 Main text

The reactivity of carbenes and metal carbenoids has enabled the facile disconnection of C–C—most notably via cyclopropanation of alkenes or C–H insertion—and C–X bonds for decades. Although the feasibility of these transformations is well established and several powerful enantioselective methods for carbenoid insertion into C–H, O–H, and N–H bonds are known, there remain challenges that render this an active and important research field.[1]

Alongside efforts to identify more efficient and selective catalysts for carbene-transfer reactions, considerable attention has been paid to identifying more abundant, non-toxic, and cheap metal-based catalysts that can promote these transformations. Iron is attractive in this context because it is non-toxic and the second most abundant metal in Earth's crust. Indeed, the scope of iron-catalyzed carbene-transfer reactions has been significantly expanded since their inception. Iron porphyrins and related systems emerged as privileged catalysts in a variety of these transformations, including alkene cyclopropanations and insertions into C(sp^2)–H and C(sp^3)–H bonds. Despite these efforts, the enantioselectivities of iron-catalyzed carbene-transfer reactions are typically lower than those described for other metals. The number of reported, highly enantioselective Fe-catalyzed C–H insertions is particularly low.[2,3]

Q4 In this context, Arnold and others have pioneered non-native biocatalytic carbene-transfer reactions, starting with the cyclopropanation of styrene in 2013.[4] Since then, hemoproteins and other iron-containing enzymes have been shown to catalyze a whole range of carbene transfers (Figure 1A), often surpassing the efficiency and selectivity of existing chemocatalysts.[5] Metalloproteins combine the advantage of the activity of the metal center with the evolvability of the protein scaffold, thereby making the selectivity of these transformations inherently tunable. Moreover, protein engineering can be combined with the use of non-native metals and cofactors, including the substitution of iron with other transition metals. Compared with reactions catalyzed by the native iron-containing enzymes, iridium substitution in particular has improved the efficiency of insertions into unactivated C–H bonds and the cyclopropanation of non-conjugated alkenes.[6]

Figure 1



Q5 Biocatalytic activation of diazirines as an alternative to diazo carbene donors
(A) Selected scope of biocatalytic carbene-insertion reactions.
(B) Methods for carbenoid formation and classification of carbenes.
(C) Model reaction and biocatalytic cyclopropanation.
(D) Mechanistic investigation of diazirine activation.
(E) Scope of carbene-insertion reactions catalyzed by *Ape*Pgb GLAVRSQLL.
(F) Superposition of *M. acetovirans* protoglobin (PDB: 3ZJO; blue) and *Ape*Pgb GLAVRSQLL (khaki); the flexible loop is marked with red.

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Metal carbenoids are most often generated from diazo compounds, which are categorized according to the electronic nature of the group(s) adjacent to the carbonic carbon (Figure 1B). The toxic and unstable nature of some of these reagents can limit their applicability, especially in the cases of donor-donor and donor-only diazo compounds. Tosylhydrazones and their derivatives have ~~also~~ been shown to be stable and convenient carbene precursors via the Bamford-Stevens reaction.[7] However, in contrast to their well-established application as carbene sources for photoaffinity labeling,[8] the use of diazirines as metal-carbenoid precursors is far less common. Indeed, their application in organic synthesis is mostly limited to palladium-catalyzed migratory insertion reactions.[7] The reason for this is most likely due to the relatively stable nature of diazirines, which often require activation by UV light, high temperatures, or electrochemical methods.

In the *Journal of the American Chemical Society*, Arnold and co-workers now report biocatalytic carbene-transfer reactions employing diazirines as carbene sources.[9] The authors showed that proteins containing the native heme-iron center are capable of catalyzing the decomposition of diazirines in the absence of light. This is noteworthy because diazirines are easily prepared and generally less toxic and more stable than the corresponding diazo compounds. Furthermore, diazirine decomposition can be done in the absence of an external base and under mild conditions. This discovery has the potential to dramatically expand the utility of biocatalytic carbene-transfer reactions.

At the outset of the study, the authors tested a variant of *Rhodothermus marinus* cytochrome *c* (*Rma* cyt *c*) for its ability to catalyze the insertion of a carbene derived from aryl trifluoromethyl diazirine **1** into the B–H bond of N-heterocyclic carbene borane **2** (Figure 1C).[8] *Rma* cyt *c* was selected because it previously catalyzed this reaction by using the isomeric diazo compound as the carbene source (Figure 1A). *Rma* cyt *c* failed in the proposed transformation, but free heme in the control reaction supported the formation of trace amounts of product **3**. A

screening approach against hemoproteins was therefore initiated, and this identified a double mutant of *Aeropyrum pernix* protoglobin (*Ape*Pgb CQ) that catalyzed this carbene transfer~~transformation~~. To improve the low yield of 0.9%, Arnold and co-workers used a homology model to identify amino acids near the enzyme active site and targeted them for mutation by site-saturated mutagenesis (SSM). This led to the identification of triple mutant *Ape*Pgb LVQ, which supported the formation of **3** in 2.6% yield. More importantly, this increase was specific to carbene donor diazirine **1** as opposed to the isomeric diazo compound, confirming that diazirine decomposition is enzyme catalyzed, and took place in the absence of light.

To prove that the transfer of carbenes not substituted with an electron-withdrawing group is also possible, the authors then tested 3-phenyl-3*H*-diazirine **4a** in the cyclopropanation of acrylate **5** given that this reaction permitted the assessment of enzyme-conferred stereoselectivity (Figure 1A). *Ape*Pgb LVQ supported the formation of the thermodynamically unfavoured *cis*-**6a** in very low yield (<1%) and with a 2:1 diastereomeric ratio (dr). Five rounds of an approach combining SSM and random mutagenesis quickly identified 9-point-mutant *Ape*Pgb GLAVRSQLL, which catalyzed the formation of *cis*-**6a** in 64% yield and with an improved 9.6:1 dr and 81:19 enantiomeric ratio (er) for the major diastereomer. This result also demonstrates the power of directed evolution to improve activity and selectivity. A study of the substrate scope, which probed the electronic effect of the aromatic substituent α to the carbonic carbon, showed that only moderately bulky and electron-withdrawing substituents were tolerated (Figure 1C). Indeed, 3-*p*-methoxyphenyl-3*H*-diazirine **4d** was not an active carbene donor. Similarly, 3-benzyl-3*H*-diazirine **4e** failed to support this cyclopropanation (Figure 1B). This work is highly notable because there is no literature precedent on the use of these donor-only diazirines in C–C bond-forming reactions. Further studies should nevertheless help to enable a better understanding of the mechanism of diazirine activation.

Although the exact mechanism of diazirine decomposition is unknown, it was plausible that diazirine **4a** might undergo enzyme-mediated isomerization to the corresponding diazo compounds. To probe this further, Arnold and co-workers used dibenzocyclooctyne amine **8** to capture the formed diazo compound *in situ* via a [3 + 2] cycloaddition reaction, and then they quantified the amount of stable pyrazole product **9** (and its regioisomer) (Figure 1D). They found that more of the pyrazoles formed in the presence of *Ape*Pgb GLAVRSQLL than in the presence of either free heme or heat-denatured enzyme. The authors thus suggested that enzyme-catalyzed diazirine-diazo isomerization is a possible activation mechanism.

*Ape*Pgb GLAVRSQLL catalyzed carbene transfer between 3-phenyl-3*H*-diazirine **4a** and a variety of carbene acceptors (Figure 1E). Cyclopropanation of styrene proceeded with 61% yield and a 12:1 dr with *cis*-**10** as the major isomer. N–H insertion and Si–H insertion into aniline and dimethyl(phenyl)silane gave lower yields of 15% and 5% of **11** and **12**, respectively, whereas insertion into the benzylic C–H bond of 1-methoxy-4-(methoxymethyl)benzene did not occur in an appreciable amount of **13**. Nevertheless, *Ape*Pgb GLAVRSQLL is a good starting point for further directed-evolution studies to identify improved catalysts for these X–H insertion reactions.

Finally, to shed light on structural determinants of the reaction, Arnold and co-workers determined the structure of mutant *Ape*Pgb GLAVRSQLL by microcrystal electron diffraction (PDB: 7UTE). In addition to mutations adjacent to the heme cofactor, the V63R, V60A, and G61V mutations were shown to greatly reorganize part of the B loop in comparison with the structure of the close protoglobin homolog from *Methanosarcina acetivorans* (Figure 1F; PDB: 3ZJO).[10] This disruption opens the protoglobin active site, making the approach and binding of carbene donors and acceptors feasible.

Less than a decade after the first reported insertion of a non-native enzymatic carbene,[4] not only has biocatalysis become well established in the field of carbene-transfer reactions, but in many ways it has also surpassed and set the standard for chemocatalytic systems. The work discussed herein describes the first enzyme-catalyzed activation of diazirines, which serve as convenient precursors to metal carbenoids, thereby allowing the insertion of a much wider variety of carbenes into N–H, Si–H, and B–H bonds. Compared with existing methods, the catalytic activation occurs under extremely mild conditions, thereby enabling the wider application of biocatalytic carbene transfer in the future.

Declaration of interests

The authors declare no competing interests.

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