Engineered Myoglobin Catalysts for Asymmetric Intermolecular Cyclopropanation Reactions

Department of Chemistry, University of Rochester, Rochester, New York 14627, United States
Mary G. Siriboe and Rudi Fasan*

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Biocatalysis has covered an increasingly important role in the synthesis and manufacturing of pharmaceuticals and other high value compounds. In the interest of expanding the range of synthetically useful reactions accessible via biocatalysts, our group has explored the potential and application of engineered myoglobins for 'abiological' carbene transfer catalysis. These transformations provide a direct route for the construction of new carbon-carbon and carbon-heteroatom bonds, including the synthesis of cyclopropane rings, which are key motifs and pharmacophores in many drugs and bioactive natural products. In this award article, we survey the progress made by our group toward the development of myoglobin-based catalysts for asymmetric intermolecular cyclopropanation reactions. The high stereoselectivity exhibited by these biocatalysts in these reactions, combined with their broad substrate scope, scalability, and robustness to high substrate loading and organic co-solvents, contribute to make these systems particularly useful for chemical synthesis and biocatalysis at the preparative scale. Extension of the scope of biocatalytic carbene transfer reactions to include different classes of carbene donor reagents has created new opportunities for the asymmetric synthesis of functionalized cyclopropanes. Furthermore, the integration of myoglobin-catalyzed stereoselective cyclopropanations with chemical diversification of the enzymatic products has furnished attractive chemoenzymatic strategies to access a diverse range of optically active cyclopropane scaffolds of high value for drug discovery, medicinal chemistry, and the synthesis of natural products.

1. Introduction

Optically active cyclopropanes are highly valuable structural motifs in medicinal chemistry, constituting key pharmacophores in several drug molecules such as the antidepressant tranylcypromine and anticoagulant drug ticagrelor.[1] Cyclopropane rings are also present in several biologically active natural products, including the insecticides permethrin and phenothrin. Because of their unique structural and conformational properties along with their established value for the design of bioactive molecules, extensive research has been devoted to the development of catalytic methods for the asymmetric construction of cyclopropane rings.[2] In this context, a direct method for the synthesis of cyclopropanes involves the metal-catalyzed cyclopropanation of olefins in the presence of diazo compounds as carbene donors.[2] These transformations involve the metal-catalyzed decomposition of the diazo compound (or an equivalent carbene donor reagent) to give rise to a reactive metallocarbenoid species, which then mediates the insertion of the carbene moiety into the C=C double bond of the olefin (**Fig. 1a**). Among others, chiral dirhodium complexes, ruthenium (or copper) bisoxazoline complexes, cobalt-salen complexes, and metalloporphyrins (**Fig. 1b**), have represented valuable catalytic systems for the realization of asymmetric cyclopropanation reactions.[2] Despite this progress, several outstanding challenges remain in this field in particular as related to achieving high stereoselectivity, catalytic efficiency, and/or overcoming the need for precious and toxic metals in these systems.

Complementing chemocatalysis, biocatalysis has covered an increasingly important role in chemical synthesis and manufacturing in both academia and industry.[3] Most attractive features of biocatalysis are the potential for high chemo-, regio- and stereoselectivity offered by enzyme-catalyzed reactions, the application of mild reaction conditions, and its sustainable nature.[3] As a result, biocatalytic processes have been implemented for the synthesis and manufacturing of a growing number of the pharmaceuticals and other high value compounds.[3a, 4] While various enzyme classes has now become integral part of the catalytic toolbox available for asymmetric synthesis, the reaction scope of naturally occurring enzymes is inherently limited compared to that of chemical methods.

Corresponding Author: Rudi Fasan
Address: Department of Chemistry, University of Rochester, New York 14627, United States
Tel: 585-273-3504
Email: rfasan@ur.rochester.edu
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In an effort toward expanding the range of synthetically useful transformations accessible via biocatalysis, our group has been exploring the design and development of engineered hemoproteins and other metalloenzymes for 'abiological' group transfer reactions, such as nitrene and carbene transfer reactions.[3e, 3f] This work led to the identification of myoglobin (**Fig. 1c**) as a particularly promising and versatile scaffold for selective carbene transfer reactions, including olefin cyclopropanation. This article provides a survey of the progress made by our group toward the development of myoglobin-based catalysts for asymmetric intermolecular cyclopropanation reactions.

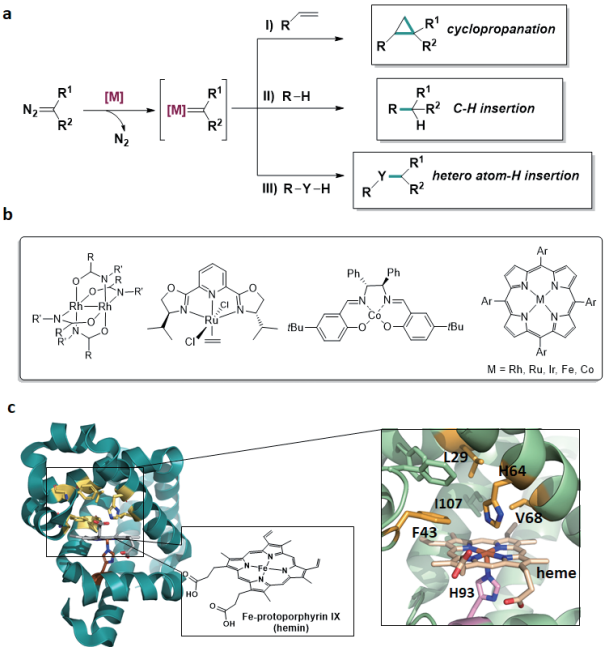


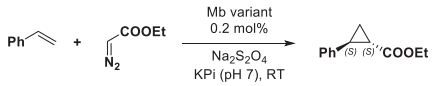
Fig. 1 Cyclopropanation via carbene transfer. (a) Cyclopropanation via transition-metal catalyzed carbene insertion into C-C bonds; (b) representative organometallic catalysts for cyclopropanation reactions via carbene transfer; (c) structure and active site of sperm whale myoglobin.

2. Biocatalytic Olefin Cyclopropanation with Diazoacetates

2. 1. Stereoselective cyclopropanation of vinylarenes

Our work on the development of engineered myoglobins for abiological carbene transfer reactions built upon our previous studies on engineering hemoproteins (cytochrome P450s, myoglobin, and others) for nitrene transfer reactions,[5] another class of synthetically useful reactions not found in nature. Inspired by this and concurrent work by the Arnold group on engineering cytochrome $P450_{BM3}$ for styrene cyclopropanation with ethyl diazoacetate (EDA),[6] our initial efforts in this area focused on exploring myoglobin as a metalloprotein scaffold for this prototypical carbene transfer reaction (**Fig. 2**).[7] While wild-type sperm whale myoglobin (Mb) catalyzes this reaction with only moderate activity and no enantioselectivity, using a rational design strategy, we were able to identify two active site mutations that can dramatically improve its activity (H64V) and its enantioselectivity (V68A) (**Fig. 2**).[7] The combination of these beneficial mutations resulted in a myoglobin catalyst, Mb(H64V,V68A), that can promote this reaction in high yields, with very high catalytic activity (>40,000 TON), and excellent diastero- and enantioselectivity for formation of the *trans*-1*S*,2*S*-configured cyclopropanation product.[7] Furthermore, this biocatalyst was found to exhibit a remarkably broad substrate scope, being able to process a broad range (~25 to date) of styrene derivatives and vinylarene substrates with high activity, high stereoselectivity, and a consistent 1*S*,2*S*-enantiopreference.[7-8] Viable substrates for this enzymatic reaction include heterocycle-containing compounds such as pyridine-, thiazole- and imidazole-based substrates (**Fig. 2**), which are notoriously challenging substrates for organometallic carbene transfer catalysts due to their ability to bind metal centers and thus inhibit the activity of these catalysts.[8] Moreover, while being atypical for enzymes, including cyclopropanation catalysts based on other metalloenzymes,[6a, 9] the combination of high activity and stereoselectivity across multiple substrates exhibited by this Mb-based catalyst contributes to make it particularly useful from a synthetic standpoint. Later studies from our group showed this 'generality' is a hallmark of Mb-based carbene transferases.[10]

In subsequent studies, the robustness of Mb(H64V,V68A) as cyclopropanation catalyst was further highlighted by demonstrating its tolerance to high reagent concentrations (e.g., up to 0.2 M styrene)[7] and to the presence of a variety of organic co-solvents,[11] which are often required in industrial biocatalysts for solubilize lipophilic substrates and/or achieve high substrate loadings.[3a, 4a, 12] For example, Mb(H64V,V68A) was shown to maintain high levels of activity and stereoselectivity in up to 30-50% (v/v) of various organic solvents, including ethanol, methanol, N,N-dimethylformamide (DMF), acetonitrile, and dimethyl sulfoxide (DMSO).[11] Furthermore, this and other Mb-based carbene transferases were shown to be amenable to long-term storage in lyophilized form, both as purified protein and as whole cells, without significant loss in activity and/or stereoselectivity.[11]



Catalyst	Yield	TON	*de*	*ee*
Mb	36%	180	86%	6%
Mb(H64V)	73%	365	92%	10%
Mb(V68A)	56%	280	96%	68%
Mb(H64V,V68A)	**>99%**	**>500**	**>99%**	**>99%**

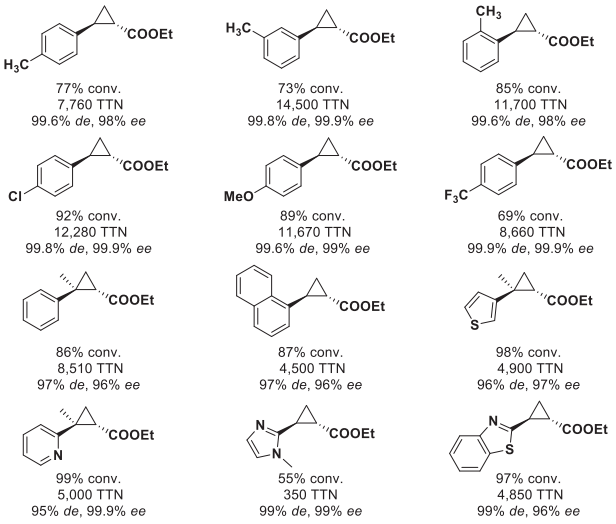


Fig. 2 Development and representative substrate scope of Mb(H64V,V68A) for *trans*-1*S*,2*S*-selective cyclopropanation of vinylarenes in the presence of EDA. TON = number of turnovers. TTN = total TON measured under catalyst-limited conditions.

Anaerobic conditions are typically required for biocatalytic cyclopropanation reactions catalyzed by heme-dependent proteins and enzymes due to oxygen binding to the heme cofactor and thus competition with the desired carbene transfer process.[3f, 6a, 7] While anaerobic conditions are useful for optimal activity of Mb(H64V,V68A) as purified protein, we determined that high yields in the cyclopropanation of styrene with EDA (68-91%) could be still achieved using whole cell reactions with *E. coli* cells expressing this enzyme.[8] In addition, we were able to simple protocols for conducting these reactions with purified protein under microaerobic or semi-aerobic conditions, which granted quantitative yields in the cyclopropanation reaction while obviating the need for strictly anaerobic conditions and specialized equipment (e.g., anaerobic chamber) for performing these transformations. Altogether, these studies demonstrate the robustness of Mb-based carbene transferases under operationally relevant conditions and provided simple protocols for application of these biocatalysts for organic synthesis.

2.2. Enantiocomplementary Cyclopropanases

The ability to access both enantiomeric forms of a target cyclopropane pharmacophore is critical in the context of the synthesis and discovery of bioactive molecules, as stereoisomers often exhibit remarkably divergent pharmacological and/or toxicity profiles.[1] However, developing enantiocomplementary variants of an enzyme is a challenging task.[13] To complement the *trans*-1*S*,2*S*-selectivity of Mb(H64V,V68A), we subsequently developed a Mb catalyst (called Mb_{RR5}) for catalyzing the cyclopropanation of styrene with EDA with inverted enantioselectivity, thus providing access to the *trans*-1*R*,2*R*-configured product of this reaction.[8] This goal was achieved after accumulation of four active mutations in the protein using a structure activity relationship (SAR)-guided protein engineering approach (**Fig. 3**). Although Mb_{RR5} is not as general as Mb(H64V,V68A) in terms of substrate scope, a broad range of aryl-substituted olefins could be cyclopropanated with high *trans*-1*R*,2*R* selectivity (78-99% *de*, 63-99% *ee*) using a small panel (5) of structurally related 'RR' variants. Using these enantiocomplementary cyclopropanation biocatalysts in combination with whole-cell biotransformations, it was possible to synthesize the chiral cyclopropane core of four drugs at the multigram scale with excellent diastereo- and stereoselectivity (98-99.9% *de*; 96-99.9% *ee*),[8] which highlights the value of these biocatalytic systems for the preparation of chiral drugs at the practical scale.

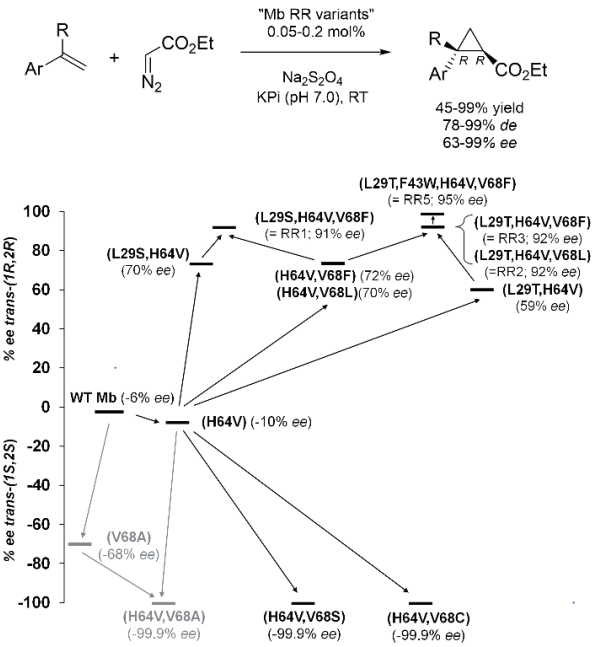


Fig. 3 SAR-guided engineering of Mb catalysts for 1*R*,2*R*-selective cyclopropanation of vinylarenes with EDA.

2. 3. Fluorinated Cyclopropanes

Owing to their peculiar effects on modulating the bioactivity potency, metabolic stability, and pharmacokinetic properties of drugs, fluorinated substituents, including mono-, di-, and trifluoromethyl groups, are extensively exploited in medicinal chemistry.[14] In spite of the well-recognized utility

of cyclopropanes and difluoromethyl (CHF_2) groups in medicinal chemistry, however, the asymmetric synthesis of difluoromethyl-containing cyclopropanes has remained largely underdeveloped.[15] To address this gap, we recently introduced a biocatalytic strategy for the stereoselective synthesis of difluoromethyl-functionalized cyclopropanes through the Mb-catalyzed cyclopropanation of α-difluoromethyl alkenes in the presence of EDA as carbene donor.[16] After screening a panel of active site Mb variants, Mb(H64V,V68A) was again identified as the best catalyst for this reaction, mediating the cyclopropanation of CHF_2-substituted styrene derivatives and other vinylarenes with good activity and high stereocontrol (93-99% *de* and 90-99% ee) for formation of the 1*R*,2*S*-configured product.[16] This stereopreference is consistent with the 1*S*,2*S*-stereoselectivity of Mb(H64V,V68A) in the reaction with styrene and EDA.[7]

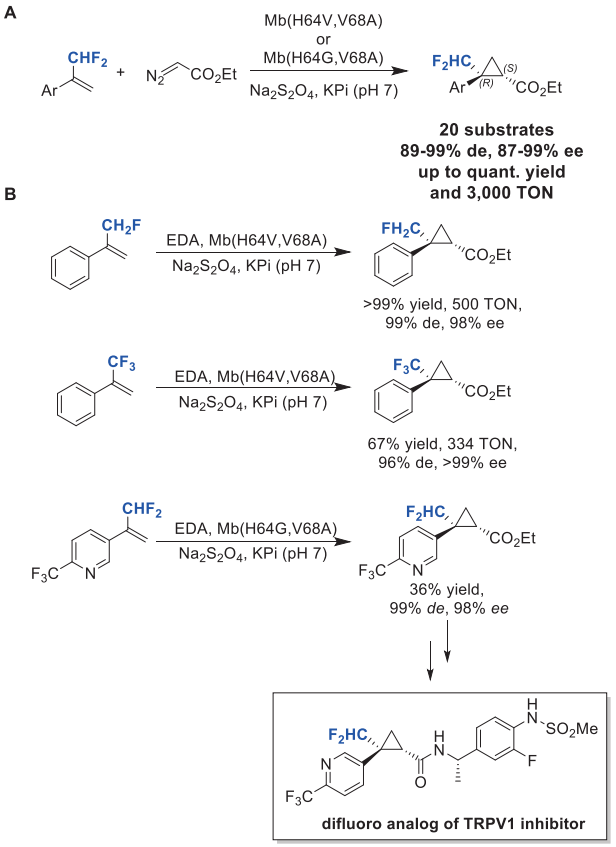


Fig. 4 Stereoselective synthesis of CHF_2-, CH_2F-, and CF_3-containing trisubstituted cyclopropanes via Mb-catalyzed carbene transfer.

In contrast to other substrates, Mb(H64V,V68A) showed modest activity and enantioselectivity of cyclopropanation of alkyl olefins and styrene derivatives with bulky substituents in the *para* position. This limitation could be overcome using a Mb variant with an enlarged cavity at the level of the distal histidine, Mb(H64G,V68A), which offered improved yields as well as higher enantioselectivity for these transformations.[16] This catalyst could be further applied for the stereoselective synthesis (99% *de*, 98% *ee*) of a difluoro analog of a TRPV1 (transient receptor potential vanilloid 1) inhibitor drug candidate developed by Pfizer.

Enantiodivergent catalysts for this transformation could be also achieved. Using a different Mb variant, namely Mb(L29T,H64V,V68F), it was indeed possible to accomplish the cyclopropanation of α-CHF_2-styrene with EDA with high stereocontrol but inverted enantioselectivity (98% de, -87% *ee*). Finally, using Mb(H64V,V68A) or Mb(H64G,V68A), the highly stereoselective cyclopropanation of monofluoromethyl- and trifluoromethyl-substituted styrene was also demonstrated (67-99% yield, 96-99% *de*, 98-99% *ee*). [16]

2. 4. Benzofuran Cyclopropanation

Benzofuran and 2,3-dihydrobenzofuran scaffolds are key structural motifs and pharmacophores in bioactive natural products and synthetic compounds.[17] The metal-catalyzed cyclopropanation of benzofurans with diazo reagents provides an attractive strategy for the preparation of 2,3-dihydrobenzofurans,[18] but the stereoselective cyclopropanation of benzofurans using readily available and inexpensive acceptor-only diazoester reagents has proven very challenging and limited to iridium-based catalysts.[17d, 17e, 19] Importantly, prior to our work, iron-based (bio)catalysts useful for promoting metallo-carbenoid cyclopropanations of benzofurans had remained elusive.

To address this gap, our group envisioned the use of engineered Mb variants to catalyze the asymmetric cyclopropanation of benzofurans with acceptor-only diazo reagents such as diazoacetates.[20] Upon screening a mini-library of Mb variants in which the shape of the heme pocket is systematically varied through rationally designed mutations, Mb(H64G,V68A) was identified as the most promising catalyst for the cyclopropanation of benzofuran with EDA (**Fig. 5**), providing excellent levels of stereoselectivity (>99.9% *de* and *ee*) along with quantitative yields under optimized conditions.[20] This reaction could be also performed in whole cells with equally high stereoselectivity and in good yields (78%). Of note, neither Fe(TPP) nor hemin were found to catalyze this reaction, highlighting the critical role of the protein matrix in conferring benzofuran cyclopropanation reactivity to the protein-embedded iron-porphyrin. Furthermore, the *trans*-selectivity of the Mb(H64G,V68A) catalyst offers a nice complement to the *cis*-selectivity of a Ir-salen catalyst previously reported by Katsuki and coworkers for a related transformation.[19a]

The scope of this methodology was demonstrated across ten different 5-, 6-, and 7- substituted and disubstituted benzofuran derivatives, all of which could be converted to the desired cyclopropanation products in good to high yields (40-99%) and with excellent diastereo- and enantioselectivity (>99% *de* and *ee*), also at a preparative scale (0.1-0.3 g).[20] This included the synthesis of an enantiopure cyclopropanation product derived from 6-bromo-benzofuran, which constitutes a key intermediate for the preparation of antidiabetic drug candidate developed by Merck. Of note, this compound could be previously afforded using state-of-the-art chemocatalytic methods only as a mixture of stereoisomers, requiring the isolation of the desired enantiomer by chiral chromatography.[17d]

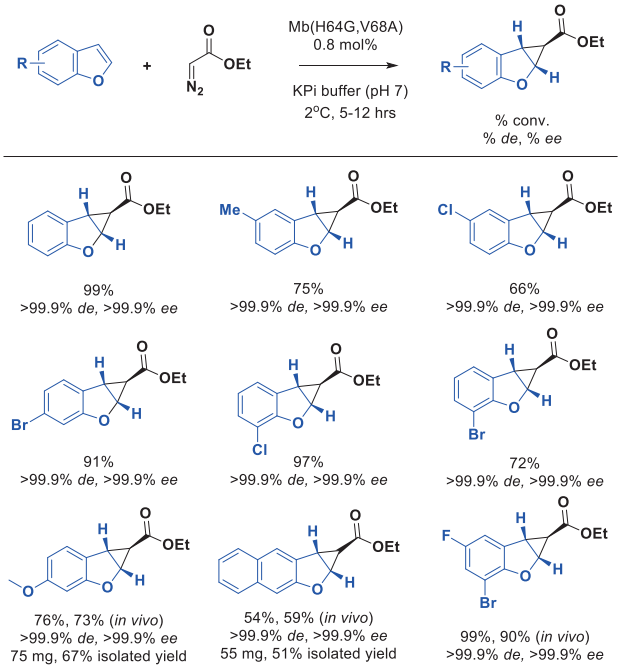


Fig. 5 Mb-catalyzed stereoselective cyclopropanation of benzofurans with ethyl diazoacetate and representative substrate scope.

By combining this methodology with our previously developed one for stereoselective cyclopropanation of vinylarenes,[7-8] it was also possible to execute a double, asymmetric cyclopropanation of 7-vinyl-benzofuran to afford a sterochemically rich tricyclic scaffold bearing five stereogenic centers in high enantiopurity (>99.9 *de* and *ee*) via a single-enzyme transformation.[20]

3. Asymmetric Cyclopropanations with Alternative Diazo Reagents

During the initial years of their development (i.e., 2013-2017), biocatalytic methods for asymmetric cyclopropanation of olefins has focused extensively on the use of diazoacetates as carbene donors.[3f] This has limited the range of cyclopropane scaffolds that can be accessed through biocatalysis. To overcome these limitations, our group has been interested in exploring other classes of carbene donors in the context of hemoprotein-catalyzed carbene transfer reactions, ultimately demonstrating the successful use of reagents such as 2-diazo-1,1,1- trifluoroethane (CF_3CHN_2),[21] diazoacetonitrile (N_2CHCN)[22] and diazoketones[23] as viable carbene precursors for myoglobin-based asymmetric cyclopropanation reactions. These methodologies have expanded the range of substituted cyclopropane motifs accessible via enzymatic catalysis and enabled the synthesis of cyclopropane rings decorated with versatile functional groups that can be further elaborated chemoenzymatically to access pharmacophores useful for the synthesis of natural products and pharmaceuticals.

3. 1. Diazotrifluoroethane

Trifluoromethyl-substituted cyclopropanes are attractive scaffolds for medicinal chemistry owing to the conformational rigidity of cyclopropane rings combined with the beneficial effect of CF_3 groups.[24] Recently, Carreira and coworkers reported the application of Co-salen catalysts for asymmetric olefin cyclopropanation with diazotrifluoroethane (DTE).[25] Inspired by this work, our group was able to develop a highly stereoselective strategy for the synthesis of trifluoromethyl-substituted cyclopropanes via cyclopropanation with DTE catalyzed by engineered myoglobin variants.[21] To enable this transformation, a compartmentalized reaction setup was implemented, in which DTE is produced *ex situ* and flown through a solution containing *E. coli* cells expressing the Mb catalyst.[21] This approach eliminated the need of isolating and handling DTE which is a highly toxic and gaseous diazo reagent. Compared to purified protein, the use of whole cells was found to grant higher yields of the enzymatic reaction, likely by protecting the catalyst from inactivation by the gas flow. Using this system, Mb(H64V,V68A) was found to be able to convert styrene derivatives and various other aryl-substituted olefins into the corresponding *trans*-(1*S*,2*S*) trifluoromethyl-substituted cyclopropane products in good to high yields (54-99%) and with high diastereo- and enantioselectivity (96-99% *de*, 92-99% *ee*) (**Fig. 6**).[21] For *para*- and *ortho*-substituted styrenes, improved diastereo- and enantioselectivity was achieved using Mb(H64V,V68G), which features an enlarged active site cavity (Val68 → Gly instead of Ala in Mb(H64V,V68A) that is beneficial to accommodate these substrates. A large-scale reaction with 100 mg of p-methoxy-styrene was also carried out, resulting in the

isolation of the corresponding CF_3-substituted cyclopropane product with an isolated yield of 76% and in excellent diastereo- and enantiomeric excess (99.9% *de*, 99.9% *ee*), demonstrating the scalability of this biocatalytic method.[21]

Interestingly, a good correlation was found between the enantiopreference of these and other Mb variants for the cyclopropanation of styrene in the presence of DTE vs. EDA.[8, 21] Leveraging this insight, trifluoromethyl-substituted cyclopropanes with a *trans*-(1*R*,2*R*) configuration could be obtained using two Mb variants, Mb(H64V,V68L,L29T) and Mb(L29T, H64V,V68F,I107L), previously developed for the 1*R*,2*R*-selective cyclopropanation of vinylarenes with EDA (98-99% *de* and 58-92% *ee*; **Fig. 6**).[21] This work represented a first example of the use of carbene donor reagents other than α-diazoesters in biocatalytic cyclopropanation reactions.

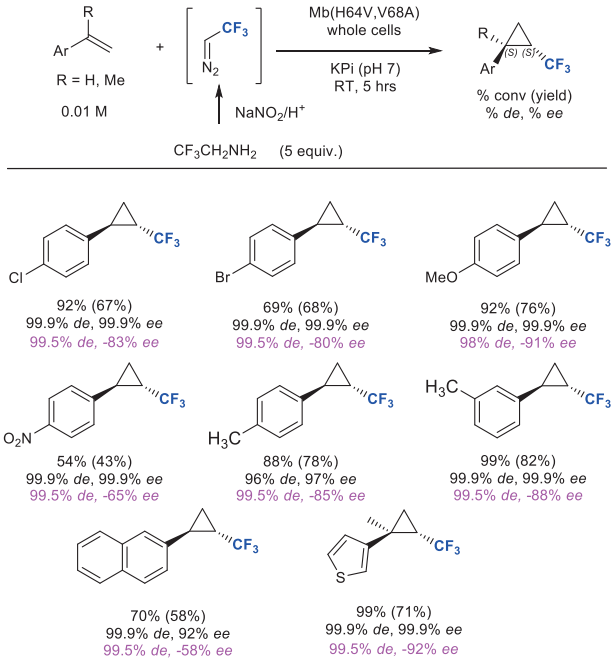


Fig. 6 Mb-catalyzed stereoselective olefin cyclopropanation with diazotrifluoroethane. Diastereo- and enantioselectivity *trans-(1S,2S)*-selective cyclopropanation using Mb(H64V,V68G) are shown in black. Diastereo- and enantioselectivity of *trans-(1R,2R)*-selective cyclopropanation using Mb(H64V,V68L,L29T) and Mb(L29T,H64V,V68F,I107L) are shown in magenta.

3. 2. Diazoacetonitrile

Nitrile substituted cyclopropanes are particularly attractive building blocks due to the versatility of the cyano group toward its interconversion to other functional groups.[26] Despite progress in the development of methods for the asymmetric synthesis of nitrile-substituted cyclopropanes using donor-acceptor or acceptor-acceptor diazocompounds,[27] the application of acceptor-only diazoacetonitrile in asymmetric cyclopropanation reactions has proven challenging.[28] To address this limitation, our group developed a general, efficient, and scalable biocatalytic strategy for the highly stereoselective synthesis of nitrile-substituted cyclopropanes via myoglobin-catalyzed cyclopropanation reactions in the presence of diazoacetonitrile.[22] Due to the volatility of this reagent, the two-part compartmentalization reaction setup previously implemented for Mb-catalyzed cyclopropanation with DTE[21] was beneficial for coupling *ex situ* diazo reagent generation with biocatalytic cyclopropanation in whole cells.[22] In initial studies, the Mb(H64V,V68A)-catalyzed cyclopropanation of *p*-chloro-styrene with diazoacetonitrile was found to produce the desired *trans*-(1*S*,2*S*)-cyclopropane product with good yield, and excellent diastereo- and enantioselectivity (55% yield, 99.9% *de* and 99.9% *ee*).[22] Upon optimization, this reaction could be carried out at the preparative scale with the improved yield of 86% and with no loss in stereoselectivity. Using this method, a diverse panel of styrene derivatives and vinylarenes were converted into the corresponding cyclopropane products in high yields (32-99%) and with high diastereo- and enantioselectivity (84-99% *de*, 76-99% *ee*) and catalytic activity (up to 5,600 TON) (**Fig. 7**). Importantly, challenging substrates including β-substituted aryl, alkyl, and electron deficient olefins, which had previously eluded Mb-catalyzed cyclopropanation with EDA, were able to undergo Mb(H64V,V68A)-catalyzed cyclopropanation with diazoacetonitrile, albeit with varying degrees of diastereo- and enantioselectivity (13-99% *de*, 1-99% *ee*) (**Fig. 7**).[22]

We envisioned that a peculiar advantage of using diazoacetonitrile as carbene precursor in biocatalytic cyclopropanation would lie in the opportunity to further diversify the enzymatic cyclopropanation product via chemical interconversion of the nitrile group. Demonstrating this point, the nitrile-functionalized cyclopropane generated via the Mb-catalyzed reaction was converted via chemical transformation (e.g., hydrolysis, reduction, or cycloaddition) of the –CN functional group to afford a diverse range of cyclopropane scaffolds in high enantiopurity (90-99% *ee*; **Fig. 8**).[22] The biocatalytic methodology described in this work offers high stereoselectivity, scalability and extends to a broad scope of olefin substrates compared to those previously amenable to Mb-catalyzed cyclopropanation with EDA, providing access to enantioenriched cyclopropane scaffolds useful for medicinal chemistry and other applications.

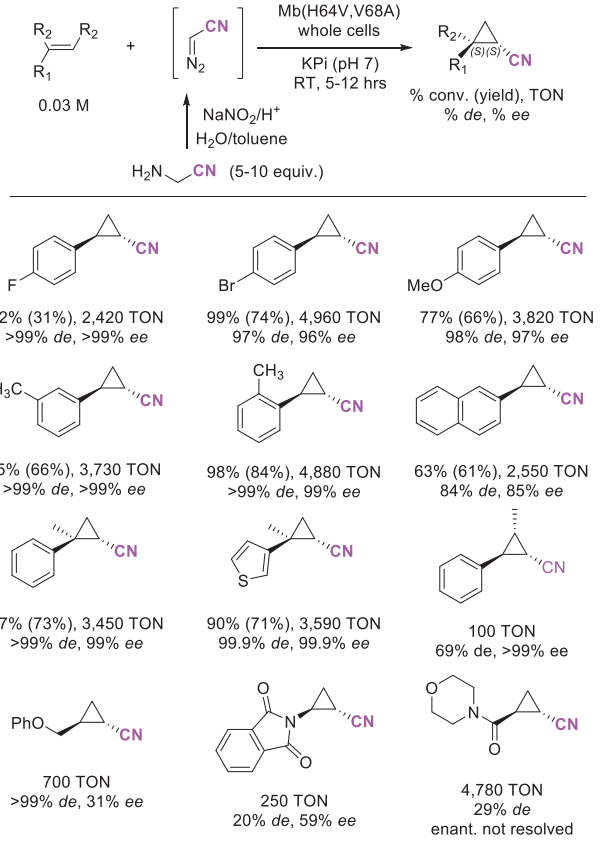


Fig. 7 Representative scope of Mb-catalyzed cyclopropanation with diazoacetonitrile.

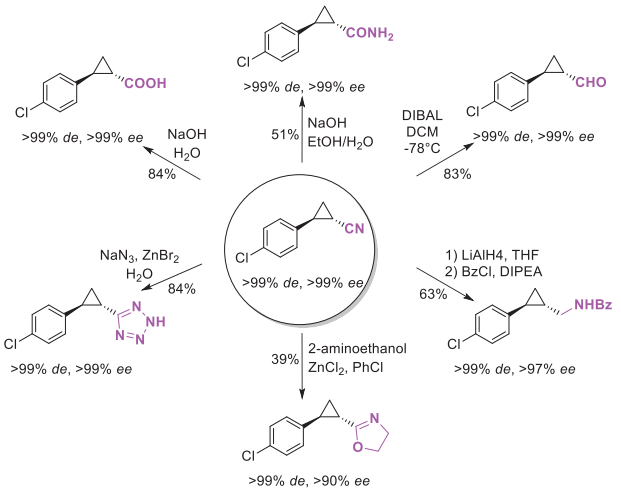


Fig. 8 Synthesis of diverse cyclopropane scaffolds via chemical elaboration of nitrile-substituted cyclopropanes generated via Mb carbene transfer catalysis.

3. 3. Benzyl diazoketones

Diazoketones are another attractive class of carbene donors which despite the versatile reactivity of carbonyl groups, has remained largely underexplored in transition metal-catalyzed carbene transfer reactions,[29] including cyclopropanations.[30] In addition, synthetic protocols for asymmetric cyclopropanations with diazoketones typically require expensive and toxic metals (Ru, Rh) and synthetically complex ligands.[29a-c, 30]

In the interest of expanding opportunities for the biocatalytic and chemoenzymatic synthesis of diverse cyclopropane scaffolds, our group recently reported the development of a biocatalytic methodology for highly stereoselective synthesis of cyclopropyl ketones via myoglobin-catalyzed cyclopropanation with 1-diazo-3-phenylpropan-2-one ('benzyl diazoketone') as the carbene donor (**Fig. 9**).[23] We envisioned the resulting cyclopropane product would offer multiple sites for chemoenzymatic diversification of the carbonyl group and α-C-H site. While wild-type Mb and various heme enzymes, including various cytochrome P450s, showed no activity in a reaction with styrene and benzyl diazoketone, Mb(H64V,V68A) showed promising activity (8% yield) along with excellent diastereo- and enantioselectivity (>99% *de*, 99% *ee*). Catalytic activity was progressively enhanced upon replacing the 'gating' distal histidine position (His64) with residues of decreasing size (i.e., Val, Ala, Gly), likely due to an increase in the accessibility of the heme pocket to diazoketone reagent. Ultimately, Mb(H64G,V68A) was identified as the best catalyst for this reaction, providing quantitative yields of the cyclopropanation product along with excellent diastereo- and enantioselectivity (>99% *de*, >99% *ee*) under optimized reaction conditions, which involved an excess of olefin over the diazo reagent in slightly alkaline buffer (pH 9).[23]

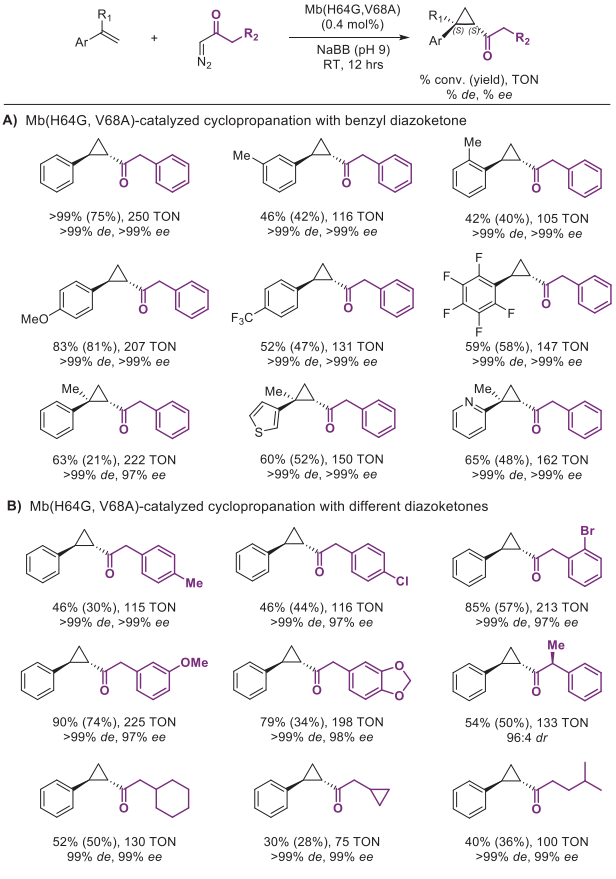


Fig. 9 Representative substrate scope of Mb-catalyzed cyclopropanation with benzyl diazoketones.

Using this catalyst, a diverse set of styrene derivatives and vinylarenes could be converted into the desired cyclopropyl ketones in moderate to high yields (21-99%) and in excellent diastereo- and enantiomeric excess (>99% *de*, 99% *ee*) (**Fig. 9A**) Additionally, the same catalyst is able to accept a variety of diazoketone reagents including *para-, meta-* and *ortho*-substituted benzyl diazoketones as well as α-alkyl-substituted diazoketones, producing the corresponding cyclopropane products with high stereoselectivity (92-99% *de*, 83-99% *ee*; **Fig. 9B**).[23]

By exploiting the unique reactivity of carbonyl group and C-H bond in alpha to it, the enzymatically produced cyclopropylketone could be further diversified through different C=O and α-C-H functionalization chemistries to afford a broad panel (10) of structurally diverse cyclopropane scaffolds in high enantiopurity, including α-cyclopropyl alcohols, amines, and fluorides, α-substituted cyclopropyl ketones, and diketones (**Fig. 10**).[23] This work illustrates the power of combining abiological biocatalysis with chemoenzymatic synthesis to generating collections of optically active scaffolds of high value for medicinal chemistry, including core motifs found in drugs and bioactive natural products.

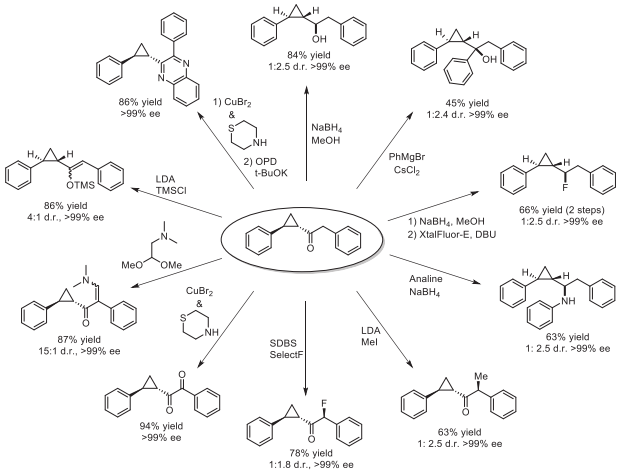


Fig. 10 Chemoenzymatic synthesis of diverse cyclopropane scaffolds enabled by Mb-catalyzed cyclopropanation with benzyl diazoketones.

4. Conclusion

Since our initial discovery of the potential of engineered myoglobins for mediating abiological group transfer reactions in 2013-2015,[5, 7, 31] this metalloprotein has provided a versatile scaffold for catalyzing a growing number of cyclopropanations and other carbene-mediated reactions with high efficiency and selectivity. The high stereoselectivity exhibited by these biocatalysts in these reactions, along with their broad substrate scope and robustness to operationally relevant conditions, contribute to make these systems particularly useful from a synthetic standpoint and for biocatalysis at the practical scale. These systems have been also instrumental in extending the reaction scope of biocatalytic carbene transfer reactions to carbene donor reagents beyond diazoesters, creating new opportunities for the asymmetric synthesis of functionalized cyclopropanes. Furthermore, the combination of myoglobin-catalyzed stereoselective cyclopropanations with chemoenzymatic synthesis has provided another attractive strategy for accessing a variety of optically active cyclopropane scaffolds of high value for drug discovery, medicinal chemistry, and the synthesis of natural products. We expect that these systems will continue to provide a fruitful source of valuable catalysts for challenging carbene transfer reactions, contributing to address the need for new, efficient, and sustainable methods for chemical synthesis.

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Profile

Rudi Fasan

Rudi Fasan is Professor of Chemistry and the Andrew S. Kende Chair of Synthetic Organic Chemistry at the University of Rochester. He earned a Master degree in Pharmaceutical Chemistry from the University of Padua (Italy) and a Ph.D. degree in Organic Chemistry from the University of Zurich (Switzerland) in 2001, working on the design and synthesis of beta-hairpin protein epitope mimetics under the supervision of Prof. John Robinson. After a postdoctoral experience with Prof. Frances Arnold at the California Institute of Technology working on the directed evolution of P450 enzymes for alkane oxidation (2005-2008), Dr. Fasan joined the Department of Chemistry at the University of Rochester, where he currently holds the position of Full Professor. In 2019, he became the inaugural recipient of the Andrew S. Kende endowed Chair in Organic and Synthetic Chemistry. His research group is engaged in the design and engineering of metalloprotein catalysts for asymmetric synthesis and 'new-to-nature' chemistry; the development of chemoenzymatic strategies for late-stage C–H functionalization; and the design and evolution of macrocyclic peptides for selective modulation of protein-protein and protein-RNA interactions. His awards include a Swiss National Science Foundation Postdoctoral Fellowship (2005-2007), the 2007 Friedrich-Weygand Outstanding Graduate Research Award, Provost Multidisciplinary Research Award (2011), University Research Award (2016, 2021), Goergen Award for Excellence in Undergraduate Teaching (2020), the 2014 Tetrahedron Young Investigator Award in Bioorganic and Medicinal Chemistry, and the 2020 JSCC International Award for Creative Work in Bioinorganic Chemistry.