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Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes

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Transition metal-catalyzed transfers of carbenes, nitrenes and oxenes are powerful methods for functionalizing C=C and C–H bonds. Nature has evolved a diverse toolbox for oxene transfers, as exemplified by the myriad monooxygenation reactions catalyzed by cytochrome P450 enzymes. The isoelectronic carbene transfer to olefins, a widely used C–C bond forming reaction in organic synthesis, has no biological counterpart. Here, we report engineered variants of cytochrome P450$_{BM3}$ that catalyze highly diastereo- and enantioselective cyclopropanation of styrenes from diazoester reagents via putative carbene transfer. This work highlights the capacity to adapt existing enzymes for catalysis of synthetically important reactions not previously observed in Nature.

The many strategies for functionalizing C=C and C–H bonds that have evolved in Nature have captivated the imaginations of chemists and form the foundation of biomimetic chemistry (*1, 2*). The reverse of this, using inspiration from synthetic chemistry to discover and develop new biocatalysts, is a nascent frontier in molecular engineering whose recent highlights include C–H activation by artificial rhodium enzymes (*3*) and the de novo design of Diels-Alderases (*4*). Synthetic chemists have developed powerful methods for direct C=C and C–H functionalization based on transition metal-catalyzed carbenoid and nitrenoid transfers, reactions that are widely used to synthesize natural product intermediates and pharmaceuticals (*5*). The asymmetric cyclopropanation of olefins with high-energy carbene precursors (e.g., acceptor-substituted diazo reagents) is a hallmark reaction that generates up to 3 stereogenic centers in a single step to make the important cyclopropane motif, featured in many natural products and therapeutic agents (*6*). Limited to using physiologically accessible reagents, Nature catalyzes intermolecular cyclopropane formation through wholly different strategies, typically involving olefin addition to the methyl cation of *S*-adenosyl methionine or through cyclization of dimethylallyl pyrophosphate-derived allylic carbenium ions (*7*). As a result, the diverse cyclopropanation products that can be formed by metallocarbene chemistry cannot be readily accessed by engineering natural cyclopropanation enzymes. We hypothesized that a natural metalloenzyme, the iron-heme-containing cytochrome P450, could be engineered to catalyze formal carbenoid transfers, thereby combining the high levels of regio- and stereoselectivity of enzymes with the synthetic versatility of carbene-based strategies.

Members of the cytochrome P450 enzyme family catalyze myriad oxidative transformations, including hydroxylation, epoxidation, oxidative ring coupling, heteroatom release, and heteroatom oxygenation (*8*). Most transformations encompassed by this broad catalytic scope manifest the reactivity of the same high-valent iron-oxene intermediate, Compound I (Fig. 1). Inspired by the impressive chemo-, regio- and stereoselectivities with which cytochrome P450s can insert oxygen atoms into C–H and C=C bonds, we investigated whether these enzymes could be engineered to mimic this chemistry for isoelectronic carbene transfer reactions via a high-valent iron-carbenoid species (Fig. 1). Here we report that variants of the cytochrome P450 from *Bacillus megaterium* (CYP102A1, or P450$_{BM3}$) are efficient catalysts for the asymmetric metallocarbene-mediated cyclopropanation of styrenes.

Because iron porphyrins catalyze carbene-based cyclopropanations (*9, 10*), we first probed whether some common heme proteins display measurable levels of cyclopropanation activity in aqueous media (phosphate buffer, 5% methanol co-solvent). We chose the reaction between styrene and ethyl diazoacetate (EDA, Fig. 2), a well-recognized model system for validating new cyclopropanation catalysts. Initial experiments showed that optimal formation of the desired cyclopropanation products occurred in the presence of a reducing agent (e.g., sodium dithionite, $Na_2S_2O_4$) under anaerobic conditions (tables S1 to S4). Horseradish peroxidase (HRP), cytochrome *c* (cyt *c*), myoglobin (Mb) and P450$_{BM3}$ all displayed multiple turnovers toward the cyclopropane products, with HRP, cyt *c* and Mb showing negligible enantioinduction and formed the *trans* cyclopropane with over 90% diastereoselectivity, which is comparable to the diastereoselectivity induced by free hemin (table S1). P450$_{BM3}$, despite forming the cyclopropane products in low yield, catalyzed the reaction with different diasteroselectivity (cis: trans 37: 63) and slight enantioinduction (Table 1), showing that carbene transfer and selectivity are dictated by the heme cofactor bound in the enzyme active site.

We then explored whether the activity and selectivity of heme-catalyzed cyclopropanation could be enhanced by engineering the protein sequence. P450$_{BM3}$ is a well-studied, soluble, self-sufficient (heme and diflavin reductase domains are fused in a single polypeptide, ~120 KDa), long-chain fatty acid monooxygenase. More than a decade of protein engineering attests to the functional plasticity of this biocatalyst (*11*). From our work using directed evolution to engineer cytochrome P450$_{BM3}$ for synthetic applications we have accumulated thousands of variants that exhibit monooxygenase activity on a wide range of substrates (*12*). We tested some of these variants for altered cyclopropanation diastero- and enantioselectivity by analysis of product distributions using gas chromatography (GC) with a chiral stationary phase. A panel of 92 P450$_{BM3}$ variants, chosen for diversity of activity and protein sequence, was screened in *E. coli* lysate for the reaction of styrene and EDA under aerobic conditions in the presence of $Na_2S_2O_4$ (tables S5 and S6). The ten most promising hits were selected for purification and characterization under standardized anaerobic reaction conditions (Table 1 and table S7).

Five of the ten selected P450s showed improvements in activity compared to wild type (total turnover numbers (TTN) > 100), a comprehensive range of diastereoselectivities with *cis*: *trans* ratios varying from 9: 91 to 60: 40, and up to 95% enantioselectivities (table S7). For example, variant H2-5-F10, which contains 16 amino acid substitutions, catalyzes 294 total turnovers, equivalent to ~ 58% yield under these

conditions (0.2% enzyme loading with respect to EDA). This represents a 50-fold improvement over wild type $P450_{BM3}$. Furthermore, mutations affect both the diastereo- and enantioselectivity of cyclopropanation: H2-5-F10 favors the *trans* cyclopropanation product (*cis*: *trans* 16: 84) with 63% ee_{trans}, whereas H2A10, with a TTN of 167, shows reversed diastereoselectivity (*cis*: *trans* 60: 40) with high enantioselectivity (95% ee_{cis}).

We used H2A10 to verify the role of the enzyme in catalysis and identify optimal conditions (table S8 and figs. S1 and S2). Heat inactivation produced diastereo- and enantioselectivities similar to those obtained with free hemin, consistent with protein denaturation and release of the cofactor. Complete inhibition was achieved by pre-incubating the reaction mixture with carbon monoxide, which irreversibly binds the reduced P450 heme, confirming that catalysis occurs at the active site. Air inhibited the cyclopropanation reaction by about 50%, showing that dioxygen and EDA compete for reduced Fe^{II}. Cyclopropanation was also achieved with NADPH as the reductant, confirming that the activity can also be driven by the endogenous electron transport machinery of the diflavin-containing reductase domain. The presence of a reducing agent in sub-stoichiometric amounts proved essential for cyclopropanation (table S9), implying that the active species is Fe^{II} rather than the resting state Fe^{III}.

Highly active $P450_{BM3}$ variants H2A10, H2-5-F10 and H2-4-D4 have three to five active site alanine substitutions with respect to 9-10A-TS-F87V (12 mutations from $P450_{BM3}$, Supporting Online Material text), which itself shows negligible cyclopropanation activity. These variants exhibit a range of TTN, diastereoselectivity, and enantioselectivity (Table 1). To better understand how protein sequence controls P450-mediated cyclopropanation, we constructed 12 variants to assess the contributions of individual alanines to catalysis and stability [table S10 (*13*)]. T268A is key for achieving high cyclopropanation activity, and this mutation alone converts inactive 9-10A-TS-F87V into an active cyclopropanation catalyst. Variant 9-10A-TS-F87V-T268A (here called BM3-CIS) is a competent cyclopropanation catalyst (199 TTN), displays strong preference for the *cis* product (*cis*: *trans* 71: 29), forms both diastereomers with over 90% *ee*, and is as stable as wild-type $P450_{BM3}$. Other active site alanine mutations tune the product distribution. Notably, the addition of I263A to BM3-CIS reverses diastereoselectivity (*cis*: *trans* 19: 81). We also investigated the effects of similar mutations introduced in the poorly active wild type $P450_{BM3}$ (table S11). Impressively, $P450_{BM3}$-T268A, with a single mutation, is an active cyclopropanation catalyst (323 TTN, Table 1) with exquisite *trans*-selectivity (cis: trans 1: 99) and high enantioselectivity for the major diastereomer (-96% ee_{trans}, Fig. 1). Whereas BM3-CIS is a *cis*-selective cyclopropanation catalyst, identical active site mutations in wild type $P450_{BM3}$ result in a *trans*-selective enzyme (table S11), demonstrating that mutations outside of the active site also influence the stereochemical outcome.

Because the design of *cis*-selective small-molecule catalysts for diazocarbonyl-mediated cyclopropanations has proven more challenging than their *trans* counterparts (*14*), we investigated whether active site engineering of $P450_{BM3}$ could provide robust *cis*-selective water-compatible catalysts to complement existing organometallic systems (*15*). We chose five active site residues (L181, I263, A328, L437, T438) for individual site-saturation mutagenesis (*13*). The A328G, T438A, T438S and T438P variants exhibited enhanced *cis*-selectivity (table S12). Notably A328G also reversed the enantioselectivity for the *cis*-diastereomer (Table 1). BM3-CIS-T438S displayed the highest diastereo- and enantioselectivities (cis: trans 92: 8 and -97% ee_{cis}) and maintained TTN comparable to BM3-CIS (Table 1).

BM3-CIS exhibits Michaelis-Menten kinetics (fig. S3 and table S13) with relatively high K_M values for the olefin (~1.5 mM) and the diazoester (~5 mM), reflecting the lack of evolutionary pressure for this enzyme to bind these substrates. BM3-CIS exhibits a notable k_{cat} for cyclopropanation of 100 min^{-1}, comparable to the k_{cat} of many native P450s for hydroxylation, but about fifty times less than $P450_{BM3}$-catalyzed fatty acid hydroxylation (table S14). Free hemin does not exhibit saturation kinetics and displays slower initial rates than BM3-CIS (only 30 min^{-1} at 10 mM styrene and 15 mM EDA), indicating that the protein scaffold enhances k_{cat} compared to the free cofactor in solution. When used at 0.2 mol% equivalent, BM3-CIS-catalyzed cyclopropanations reached completion after 30 min. Adding more EDA enhanced turnovers for cyclopropanes and preserved BM3-CIS stereoselectivity (table S15), confirming catalyst integrity and implying that the reaction stops because of EDA depletion rather than inactivation.

To assess the substrate scope of $P450_{BM3}$-catalyzed cyclopropanation, we investigated the activities of six variants against a panel of olefins and diazo compounds (Table 2 and tables S16-S20). P450 cyclopropanation is robust to both electron-donating (*p*-vinylanisole, *p*-vinyltoluene) and electron-withdrawing (*p*-trifluoromethylstyrene) substitutions on styrene, and variant 7-11D showed consistent cis-selectivity for these substrates. The P450s were also active on 1,1-disubstituted olefins (i.e., α-methyl styrene), with chimeric P450 C2G9R1 forming cyclopropanes in 77% yield (with respect to EDA). The P450s were only moderately active with *t*-butyl diazoacetate as substrate (<30% yield), forming the *trans* product with >87% selectivity and offering no advantage over free hemin (table S20). For reactions involving EDA and aryl-substituted olefins, however, the P450s consistently outperformed the free cofactor in both activity and stereoselectivity.

Screening natural enzymes against synthetic reagents chosen based on chemical intuition offers a simple strategy for identifying enzymes with basal levels of non-native activity. As we have shown, a single mutation can be enough to promote such activity and achieve synthetically useful stereoselectivities. Accumulation of beneficial mutations by directed evolution or other protein engineering strategies can generate a spectrum of highly active catalysts for desired substrate and product specificities. The established reaction promiscuity of natural enzymes (*16*, *17*) and the surprising ease with which cyclopropanation activity could be installed into $P450_{BM3}$ suggest that this approach will be useful for other synthetically important transformations for which biological counterparts do not yet exist.

References and Notes

1. J. T. Groves, The bioinorganic chemistry of iron in oxygenases and supramolecular assemblies. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3569 (2003). doi:10.1073/pnas.0830019100 Medline
2. R. Breslow, Biomimetic chemistry: Biology as an inspiration. *J. Biol. Chem.* **284**, 1337 (2009). doi:10.1074/jbc.X800011200 Medline
3. T. K. Hyster, L. Knörr, T. R. Ward, T. Rovis, Biotinylated Rh(III) complexes in engineered streptavidin for accelerated asymmetric C-H activation. *Science* **338**, 500 (2012). doi:10.1126/science.1226132 Medline
4. J. B. Siegel *et al*., Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. *Science* **329**, 309 (2010). doi:10.1126/science.1190239 Medline
5. H. M. L. Davies, J. R. Manning, Catalytic C-H functionalization by metal carbenoid and nitrenoid insertion. *Nature* **451**, 417 (2008). doi:10.1038/nature06485 Medline
6. H. Lebel, J.-F. Marcoux, C. Molinaro, A. B. Charette, Stereoselective cyclopropanation reactions. *Chem. Rev.* **103**, 977 (2003). doi:10.1021/cr010007e Medline
7. L. A. Wessjohann, W. Brandt, T. Thiemann, Biosynthesis and metabolism of cyclopropane rings in natural compounds. *Chem. Rev.* **103**, 1625 (2003). doi:10.1021/cr0100188 Medline
8. E. M. Isin, F. P. Guengerich, Complex reactions catalyzed by cytochrome P450 enzymes. *Biochim. Biophys. Acta, Gen. Subj.* **1770**, 314 (2007). doi:10.1016/j.bbagen.2006.07.003
9. J. R. Wolf, C. G. Hamaker, J.-P. Djukic, T. Kodadek, L. K. Woo, Shape and

stereoselective cyclopropanation of alkenes catalyzed by iron porphyrins. *J. Am. Chem. Soc.* **117**, 9194 (1995). doi:10.1021/ja00141a011
10. B. Morandi, E. M. Carreira, Iron-catalyzed cyclopropanation in 6 M KOH with in situ generation of diazomethane. *Science* **335**, 1471 (2012). doi:10.1126/science.1218781 Medline
11. C. J. C. Whitehouse, S. G. Bell, L.-L. Wong, P450(BM3) (CYP102A1): Connecting the dots. *Chem. Soc. Rev.* **41**, 1218 (2012). doi:10.1039/c1cs15192d Medline
12. J. C. Lewis, F. H. Arnold, Catalysts on demand: Selective oxidations by laboratory-evolved cytochrome P450 BM3. *Chimia (Aarau)* **63**, 309 (2009). doi:10.2533/chimia.2009.309
13. Materials and methods are available as supplementary materials on *Science* Online.
14. A. Caballero, A. Prieto, M. M. Diaz-Requejo, P. J. Perez, Metal-catalyzed olefin cyclopropanation with ethyl diazoacetate: Control of the diastereoselectivity. *Eur. J. Inorg. Chem.* **2009**, 1137 (2009). doi:10.1002/ejic.200800944
15. I. Nicolas, P. Le Maux, G. Simonneaux, Asymmetric catalytic cyclopropanation reactions in water. *Coord. Chem. Rev.* **252**, 727 (2008). doi:10.1016/j.ccr.2007.09.003
16. U. T. Bornscheuer, R. J. Kazlauskas, Catalytic promiscuity in biocatalysis: Using old enzymes to form new bonds and follow new pathways. *Angew. Chem. Int. Ed.* **43**, 6032 (2004). doi:10.1002/anie.200460416
17. O. Khersonsky, C. Roodveldt, D. S. Tawfik, Enzyme promiscuity: Evolutionary and mechanistic aspects. *Curr. Opin. Chem. Biol.* **10**, 498 (2006). doi:10.1016/j.cbpa.2006.08.011 Medline
18. A. Penoni *et al*., Cyclopropanation of olefins with diazoalkanes, catalyzed by Co^{II}(porphyrin) complexes − a synthetic and mechanistic investigation and the molecular structure of Co^{III}(TPP)(CH_2CO_2Et) (TPP = dianion of meso-tetraphenylporphyrin). *Eur. J. Inorg. Chem.* **2003**, 1452 (2003). doi:10.1002/ejic.200390189
19. N. Watanabe, H. Matsuda, H. Kuribayashi, S.-i. Hashimoto, Dirhodium(II) tetrakis[3(S)-phthalimido-2-piperidinonate]: A novel dirhodium(II) carboxamidate catalyst for asymmetric cyclopropanation. *Heterocycles* **42**, 537 (1996). doi:10.3987/COM-95-S94
20. J. Sambrook, E. Frisch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, New York, 1989), vol. 2.
21. C. R. Otey, in *Methods in Molecular Biology: Directed Enzyme Evolution*, F. H. Arnold, G. Georgiou, Eds. (Humana Press, Totowa, NJ, 2003), vol. 230.
22. M. W. Peters, P. Meinhold, A. Glieder, F. H. Arnold, Regio- and enantioselective alkane hydroxylation with engineered cytochromes P450 BM-3. *J. Am. Chem. Soc.* **125**, 13442 (2003). doi:10.1021/ja0303790 Medline
23. A. Glieder, E. T. Farinas, F. H. Arnold, Laboratory evolution of a soluble, self-sufficient, highly active alkane hydroxylase. *Nat. Biotechnol.* **20**, 1135 (2002). doi:10.1038/nbt744 Medline
24. P. Meinhold, M. W. Peters, A. Hartwick, A. R. Hernandez, F. H. Arnold, Engineering cytochrome P450 BM3 for terminal alkane hydroxylation. *Adv. Synth. Catal.* **348**, 763 (2006). doi:10.1002/adsc.200505465
25. R. Fasan, M. M. Chen, N. C. Crook, F. H. Arnold, Engineered alkane-hydroxylating cytochrome P450BM3 exhibiting nativelike catalytic properties. *Angew. Chem. Int. Ed.* **46**, 8414 (2007). doi:10.1002/anie.200702616
26. J. C. Lewis *et al*., Combinatorial alanine substitution enables rapid optimization of cytochrome P450BM3 for selective hydroxylation of large substrates. *ChemBioChem* **11**, 2502 (2010). doi:10.1002/cbic.201000565 Medline
27. J. C. Lewis *et al*., Chemoenzymatic elaboration of monosaccharides using engineered cytochrome P450BM3 demethylases. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 16550 (2009). doi:10.1073/pnas.0908954106 Medline
28. C. R. Otey *et al*., Structure-guided recombination creates an artificial family of cytochromes P450. *PLoS Biol.* **4**, e112 (2006). doi:10.1371/journal.pbio.0040112
29. M. Landwehr, M. Carbone, C. R. Otey, Y. Li, F. H. Arnold, Diversification of catalytic function in a synthetic family of chimeric cytochrome p450s. *Chem. Biol.* **14**, 269 (2007). doi:10.1016/j.chembiol.2007.01.009 Medline
30. M. M. Chen, P. S. Coelho, F. H. Arnold, Utilizing terminal oxidants to achieve P450-catalyzed oxidation of methane. *Adv. Syn. Cat.* **354**, 964 (2012). doi:10.1002/adsc.201100833
31. M. A. Noble *et al*., Roles of key active-site residues in flavocytochrome P450 BM3. *Biochem. J.* **339**, 371 (1999). doi:10.1042/0264-6021:3390371 Medline
32. R. Fasan, Y. T. Meharenna, C. D. Snow, T. L. Poulos, F. H. Arnold, Evolutionary history of a specialized p450 propane monooxygenase. *J. Mol. Biol.* **383**, 1069 (2008). doi:10.1016/j.jmb.2008.06.060 Medline
33. C. J. Sanders, K. M. Gillespie, P. Scott, Catalyst structure and the enantioselective cyclopropanation of alkenes by copper complexes of biaryldiimines: The importance of ligand acceleration. *Tetrahedron Asymmetry* **12**, 1055 (2001). doi:10.1016/S0957-4166(01)00157-4
34. M. L. Rosenberg, A. Krivokapic, M. Tilset, Highly *cis* -selective cyclopropanations with ethyl diazoacetate using a novel Rh(I) catalyst with a chelating N-heterocyclic iminocarbene ligand. *Org. Lett.* **11**, 547 (2009). doi:10.1021/ol802591j Medline
35. Y. Chen, X. P. Zhang, Asymmetric cyclopropanation of styrenes catalyzed by metal complexes of D2-symmetrical chiral porphyrin: Superiority of cobalt over iron. *J. Org. Chem.* **72**, 5931 (2007). doi:10.1021/jo070997p Medline

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Supplementary Materials

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Supplementary Text
Figs. S1 to S3
Tables S1 to S20
References (*18*–*35*)

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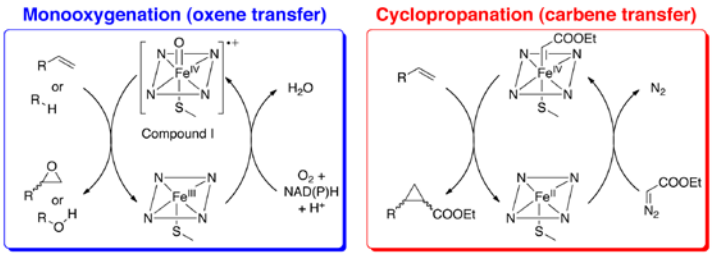

Fig. 1. Canonical mode of reactivity of cytochrome P450s (**left**): Monooxygenation of olefins and C-H bonds to epoxides and alcohols catalyzed by the ferryl porphyrin radical intermediate (compound I). Artificial mode of formal carbene transfer activity of cytochrome P450s utilizing diazoester reagents as carbene precursors (**right**).

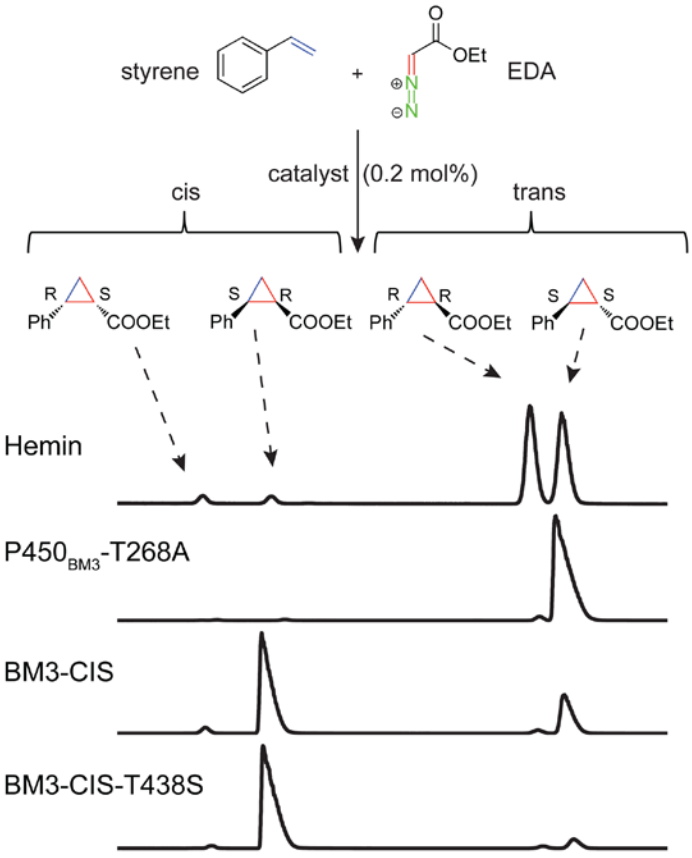

Fig. 2. Absolute stereoselectivity of select $P450_{BM3}$ cyclopropanation catalysts. Reaction conditions: 20 μM catalyst, 30 mM styrene, 10 mM EDA, 10 mM $Na_2S_2O_4$, under argon in aqueous potassium phosphate buffer (pH 8.0) and 5% MeOH cosolvent for 2 hours at 298 K. Enzyme loading is 0.2 mol % with respect to EDA. The structures of each product stereoisomer are shown above the reaction gas chromatograms.

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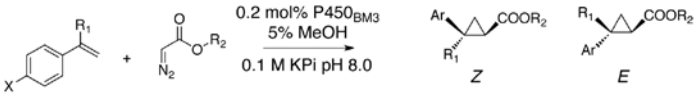
Table 1. Stereoselective $P450_{BM3}$ cyclopropanation catalysts. Reactions were run in aqueous phosphate buffer (pH 8.0) and 5% MeOH cosolvent at room temperature under argon with 30 mM styrene, 10 mM EDA, 0.2 mol % catalyst (with respect to EDA), and 10 mM $Na_2S_2O_4$. Yields, diastereomeric ratios, and enantiomeric excess were determined by GC analysis. Yields based on EDA. TTN = total turnover number. *(*R*,*S*) – (*S*,*R*). †(*R*,*R*) – (*S*,*S*). See supplementary text for protein sequences indicating mutations from wild type $P450_{BM3}$.

Catalyst	**% yield**	**TTN**	**cis: trans**	**%ee_{cis}***	**%ee_{trans}†**
Hemin	15	73	6: 94	-1	0
$P450_{BM3}$	1	5	37: 63	-27	-2
$P450_{BM3}$ -T268A	65	323	1: 99	-15	-96
9-10A-TS-F87V	1	7	35: 65	-41	-8
H2-5-F10	59	294	16: 84	-41	-63
H2A10	33	167	60: 40	-95	-78
H2-4-D4	41	206	53: 47	-79	-33
BM3-CIS	40	199	71: 29	-94	-91
BM3-CIS-I263A	38	190	19: 81	-62	-91
BM3-CIS-A328G	37	186	83: 17	52	-45
BM3-CIS-T438S	59	293	92: 8	-97	-66

Table 2. Scope of P450 catalyzed cyclopropanation of styrenyl substrates. Ar = *p*-X-C_6H_4. Reaction conditions: 20 μM catalyst, 30 mM olefin, 10 mM diazoester, 10 mM $Na_2S_2O_4$, under argon in aqueous potassium phosphate buffer (pH 8.0) and 5% MeOH cosolvent for 2 hours at 298 K. Enzyme loading is 0.2 mol % with respect to diazoester. N/A = not available when enantiomers did not separate to baseline resolution.



Reagents	P450 catalyst	TTN	*Z* : *E*	%ee_Z	%ee_E
R_1 = H, X= Me, R_2 = Et	BM3-CIS	228	78 : 22	-81	N/A
R_1 = H, X= OMe, R_2 = Et	H2-5-F10	364	11 : 89	38	N/A
R_1 = H, X= CF_3, R_2 = Et	7-11D	120	76 : 24	31	59
R_1 = Me, X= H, R_2 = Et	7-11D	157	41 : 49	42	N/A
R_1 = H, X= H, R_2 = *t*-Bu	H2A10	120	3 : 97	N/A	N/A