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Myoglobin-catalyzed olefination of aldehydes

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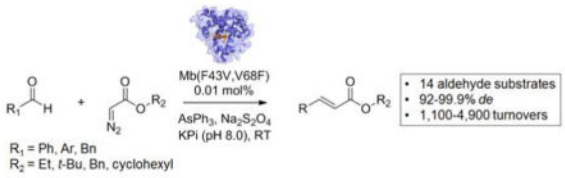
Abstract

The olefination of aldehydes constitutes a most valuable and widely adopted strategy for constructing carbon-carbon double bonds in organic chemistry. While various synthetic methods have been made available for this purpose, no biocatalysts are known to mediate this transformation. Here, we report that engineered myoglobin variants can catalyze the olefination of aldehydes in the presence of α-diazoesters with high catalytic efficiency (up to 4,900 turnovers) and excellent E-diastereoselectivity (92–99.9% de). This transformation could be applied to the olefination of a variety of substituted benzaldehydes, heteroaromatic and alkyl aldehydes, also in combination with different alkyl α-diazoacetate reagents. This work provides a first example of biocatalytic aldehyde olefination and extends the spectrum of synthetically valuable chemical transformations accessible using metalloprotein-based catalysts.

Graphical Abstract



Biocatalytic Wittig olefination: Engineered variants of sperm whale myoglobin can serve as biocatalysts for the conversion of aldehydes and α-diazo esters to the corresponding α,β-unsaturated esters. This transformation proceeds with high catalytic efficiency and high *E*-diastereoselectivity and could be applied to a variety of different aldehyde substrates and α-diazo-esters.

Keywords

Biocatalysis; Wittig reaction; myoglobin; carbene transfer; protein engineering; aldehyde olfination

The Wittig reaction[1] represents one of the most valuable and broadly adopted route for the construction of olefinic bonds during the synthesis of organic molecules.[2] Classically, this

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method involves the reaction between carbonyl compounds and phosphonium ylides, which are prepared by deprotonation of the corresponding phosphonium salts[3]. Because of the basic conditions required for the latter process, there has been a significant interest toward developing alternative methods to enable the olefination of aldehydes under milder, neutral conditions. In this regard, the transition metal catalyzed transformation of carbonyls in the presence of diazo compounds and tertiary phosphines has provided a particularly attractive strategy due to the readily accessibility of these reagents.[4] Over the recent years, a number of organometallic catalysts including Mo[4a], Re[4b–d], Rh[4e], Ir[4f], Ru[4g, 4h], Cu[4i] and Fe[4j–o] complexes have proven useful in this transformation, yielding *E*-olefins with modest to good catalytic activity (typically, 50–300 turnovers) and moderate to high *E*-selectivity (typically, 70–98% *de*). In contrast to the important progress made in the development of synthetic catalysts for aldehyde olefination, no natural enzyme or artificial biocatalysts[5] has been reported to promote this valuable transformation. An aldehyde olefination biocatayst would thus represent a valuable addition to the toolbox of currently available enzymes for asymmetric synthesis[6].

We and others have recently reported the ability of heme-dependent metalloproteins such as cytochrome P450s[7] and myoglobin[8] to engage diazo-containing reagents in carbene transfer reactions. In particular, we recently discovered that engineered variants of myoglobin can provide particularly efficient catalysts for olefin cyclopropanation[8a], carbene N–H insertion[8b], and carbene S–H insertion reactions[8c] in the presence of α-diazo ester reagents. Our mechanistic studies supported the intermediacy of an electrophilic heme-carbene complex[8a] which reacts with a nucleophilic olefin, amine or mercaptan to yield the carbene insertion adduct. These studies also showed the possibility to generate a transient sulfonium ylide intermediate upon attack of a thiol substrate to the myoglobin-bound carbenoid species.[8c] Building upon these findings and inspired by pioneering studies conducted by Woo and coworkers with metalloporphyrins[4j, 4l], we hypothesized that an analogous process could be exploited in the presence of tertiary phosphine nucleophiles to yield a myoglobin-bound phosphonium ylide. We further envisioned the latter could react with an aldehyde to yield an olefin via a Wittig reaction, with the active site of the protein potentially furnishing an asymmetric environment to influence the stereoselectivity of the reaction. Here, we report that engineered variants of myoglobin can mediate aldehyde olefination reactions across a range of aldehydes and α-diazoacetates with high catalytic activity and *E*-selectivity. This transformation proceeds in buffer and at room temperature, thus providing an extremely mild biocatalytic route for the olefination of aryl and alkyl aldehydes.

Guided by the hypothesis outlined above, we began our studies by testing the ability of wild-type sperm whale myoglobin to promote the conversion of benzaldehyde **1a** and ethyl α-diazo acetate (EDA, **2a**) to ethyl cinnamate **3a** in the presence of triphenylphosphine (PPh_3). To our delight, we observed formation of the desired product **3a** with good diastereoselectivity (76% *de* for *E*-isomer), albeit with only modest activity (31 turnovers or TON) (Table 1, Entry 3). Both reducing ($Na_2S_2O_4$) and oxygen-free conditions were found to be required for the observed Mb-dependent aldehyde olefination activity, consistent with the idea that the ferrous form of the hemoprotein is involved in the activation of the diazo

compound. Additional experiments showed that hemin can also promote this transformation, but with reduced catalytic efficiency (22 TON) and lower diastereoselectivity (65% *de*) as compared to Mb (Table 1, Entry 1). In addition, the hemin reaction is much less chemoselective, yielding larger amounts of the carbene dimerization byproducts, diethyl fumarate and diethyl maleate ($TON_{(\mathbf{3a})}/TON_{(\mathbf{4a})}$: 0.4 vs. 2.8 with Mb, Table 1). In an effort to improve the efficiency and selectivity of the Mb-mediated olefination reaction, a variety of trialkyl phosphines (e.g., PEt_3, P(*t*-Bu)$_3$, P(*n*-Bu)$_3$) as well as heavier congeners of PPh_3 (i.e., $AsPh_3$, $AsPh_3$, and $BiPh_3$) were tested as a substitute for triphenylphosphine (Table 1). Interestingly, whereas neither $BiPh_3$ nor any of the trialkyl phosphines led to the desired olefin product, the reaction in the presence of $AsPh_3$ exhibited excellent diastereoselectivity, leading to the formation of *trans*-**3a** as the only detectable isomer (>99.9% *de*).

Encouraged by these results, we extended our investigations to a panel of Mb variants containing one or two mutations at the level of the protein active site. In sperm whale Mb, five amino acid residues (Leu29, Phe43, His64, Val68, Ile107) define the cavity located above the distal face of the heme cofactor (Figure S1).[9] Previously, we found that mutagenesis of these residues had a profound impact on the selectivity and activity of Mb-catalyzed carbene transfer reactions.[8] Accordingly, Mb active site variants were tested for their relative activity and selectivity in the olefination of benzaldehyde with EDA in the presence of either PPh_3 or $AsPh_3$. As summarized in Table 2, the active site mutations were found to have noticeable effects on the catalytic efficiency (TON), diastereoselectivity, and chemoselectivity of the reaction. Among the Mb variants tested, the double mutant Mb(F43V,V68F), used in combination with $AsPh_3$, emerged as the most promising catalyst for this reaction, exhibiting 3-fold higher TON compared to wild-type Mb, excellent diasteroselectivity (>99.9% *de*), and high chemoselectivity toward aldehyde olefination over carbene dimerization. At a catalyst loading of 0.01 mol%, Mb(F43V,V68F) was determined to support over 1,100 catalytic turnovers for the conversion of **1** to ***E*-3a**, featuring an initial rate of 320 and 40 turnovers min^{-1} over the first minute and first 15 minutes, respectively (Figure S2). Importantly, nearly absolute *E*-selectivity as well as high chemoselectivity ($TON_{(olefin)}$:$TON_{(dimer)}$ > 4) are maintained under these conditions, the latter being achieved without the need for slow addition of the diazo compound as typically required for metalloporphyrin catalysts[4h, 4j, 4n] in order to minimize carbene dimerization.

Across nearly all Mb variants, the $AsPh_3$-supported reactions consistently furnished higher degrees of diastereoselectivity as compared to those performed in the presence of PPh_3 (Table 2). The only exception was Mb(H64V,V68A), for which a reversal of this trend was observed (70% vs. 57% *de* for reaction with PPh_3 vs. $AsPh_3$). Intriguing is also the differential effect of the active site mutations in the context of this reaction as compared to the carbene-mediated transformations previously investigated by our group.[8] For example, while the double mutation in Mb(H64V,V68A) greatly enhanced the reactivity and selectivity of Mb toward olefin cyclopropanation,[8a] the same mutations led to a reduction in TON, diastereo- and chemoselectivity for the aldehyde olefination reaction (Entry 9 vs. 1, Table 2). These differences highlight the peculiar active site requirements for favouring high reactivity and selectivity in the context of these related yet mechanistically distinct reactions.

To investigate the scope of Mb(F43V,V68F) as aldehyde olefination catalyst, the reaction with benzaldehyde was carried out in the presence of other α-diazo esters, including *tert*-butyl (**2b**), benzyl (**2c**), and cyclohexyl (**2d**) α-diazo acetate as well as ethyl α-diazo-propanoate (**2e**). Notably, despite their variable alkyl chain, all of the α-diazo-acetates (**2b**–**2d**) could be readily processed by the biocatalyst to yield the corresponding *trans* β-aryl-α,β-unsaturated ester products, **3b**–**3d**, with good (79–83% *de*) to excellent (98–99.9% *de*) selectivity in the presence of PPh_3 and $AsPh_3$, respectively (Table 3). In combination with **3c**, Mb(F43V,V68F) gave the highest TON value (4,920) and conversion ratio (49%), whereas the use of the Mb(F43V,V68F)/**3d**/$AsPh_3$ system provided an optimal combination of high catalytic activity (4,200 TON) with excellent stereocontrol (99.9% *de*). As such, the latter system was maintained for further studies on the scope of this hemoprotein across different aldehydes (*vide infra*). Under these optimized conditions, the TON values supported by Mb(F43V,V68F) in water and at room temperature are one to two orders of magnitudes higher than those previously reported for similar transformations catalyzed by organometallic complexes in organic solvent and at elevated temperature (50–300 TON[4a–k, 4m, 4n]). The only exception is the Fe(TPP)Cl-catalyzed olefination of benzaldehyde with EDA and PPh_3 in toluene at 80°C reported by Zhang and coworkers, for which even higher TON (8,900) but also lower diastereoselectivity (84% *de*) were measured.[4h] In contrast to **2b–2d**, no olefination product was observed in the presence of **2e**, indicating that α-substitutions on the carbene moiety are not tolerated by the Mb catalyst in the context of this reaction.

Next, the scope of Mb(F43V,V68F)-catalyzed olefination across different aldehyde substrates was investigated. As summarized in Scheme 1, a variety of monosubstituted benzyaldehyde derivatives (**5a**–**13a**) could be readily converted to the corresponding cyclohexyl *trans*-cinnamate esters **5b**–**13b** with very good to excellent diastereoselectivity (99–99.9% *de*), with the Mb catalyst supporting from 1,110 (**13b**) to 3,400 turnovers (**5b** and **7b**). Insights into the impact of electronic factors on the efficiency of the reaction could be gained from side-by-side comparison of the TON values for benzaldehyde derivatives carrying substituents of similar size but with different electronic properties. In particular, electron-deficient benzaldehydes were found to be consistently less reactive than their isosteric, electron-richer counterparts, as indicated by the lower TON measured for **8b** vs. **3b**, for **11b** vs. **6b**, and **9b** vs. **7b**. This trend contrasts with the higher reactivity of electron-poor aldehydes in transition metal-catalyzed olefination reactions in organic solvents[4h, 4j] and it can be rationalized on the basis of the higher level of hydration expected for benzaldehydes carrying electronwithdrawing groups in water.[10] A higher degree of hydration is expected to reduce the effective concentration of aldehyde susceptible to nucleophilic attack by the phoshonium ylide (*vide infra*), thus reducing the overall efficiency of the reaction.

The sucessful conversion of **14a** to **14b** showed that disubstituted benzyaldehydes could be also processed by the Mb(F43V,V68F) catalyst, albeit with lower efficiency (1,140 vs. 3,400 TON) and selectivity (91% vs. 98% de) compared to the monosubstituted counterpart, **5b**. Substrates such as 2-naphthaldehyde (**15a**) and thiophene-2-carbaldehyde (**16a**) could also be converted to the corresponding *trans* olefin products **15b** and **16b**, respectively, with

excellent selectivity (99% *de*), further supporting the broad substrate scope of Mb(F43V,V68F) across structurally different aryl aldehydes. Finally, the successful olefination of phenylacetaldehyde (**17a**) to give **17b** (1,940 TON; 92% *de*) demonstrated the reactivity of the catalyst also toward alkyl aldehydes.

A proposed mechanism for the Mb-catalyzed aldehyde olefination reaction is presented in Scheme 2. Starting from the catalytically active ferrous form of the hemoprotein, the first step is envisioned to involve the formation of a heme-bound carbenoid intermediate (II in Scheme 2) upon reaction with the diazo reagent. This intermediate can be formally described as an iron(IV)-carbene complex or as a Fe^{II}←{:$CHCO_2R$} complex, the latter being predicted to be a more stable resonance form at least in the context of synthetic iron-porphyrin systems.[11] Regardless of its exact nature, our previous studies showed that this species has electrophilic character[8a] and can react with thiol nucleophiles to generate a transient sulfonium ylide.[8c] Accordingly, attack of the nucleophilic PPh_3 (or $AsPh_3$) to the heme-carbene intermediate is envisioned to ensue giving rise to a phosphonium ylide (species III, Scheme 2). The latter would then react with the aryl aldehyde to generate a oxaphosphetane intermediate[12] (species IV, Scheme 2), whose rearrengement yields the olefin product and phosphine oxide as the byproduct.

In view of the mechanistic model of Scheme 2, a number of considerations can be made in regard of the results described earier. A first one concerns the role of the biocatalyst on influencing the stereoselectivity of the reaction. Importantly, the Mb reaction with EDA and PPh_3 in the absence of aldehyde was found to accumulate the phosphorane intermediate (Ph_3P=$CHCO_2Et$) in solution, thus supporting the occurrence of the steps I → II → III proposed in Scheme 2. Insightfully, a reaction of premade phosphorane with benzaldehyde yielded ***E*-3b** in 80% *de* both in the presence and in the absence of the Mb catalyst. Such diastereoselectivity differs from that observed in the olefination reactions with nearly all of the Mb variants starting from benzaldehyde and EDA (64–76% *de*, Table 2). This result together with the higher diastereoselectivity obtained with Mb vs. hemin (Table 1) and the effect of active site mutations on the *E* : *Z* ratio of the olefin product (Table 2) support the involvement of the protein environment in affecting the stereochemical outcome of the reaction. Since the stereoselectivity of the Wittig reaction is largely dictated by the relative orientation of the ylide and aldehyde during formation of the oxaphosphetane intermediate,[2b, 2c, 12b, 15] the asymmetric induction imposed by the hemoprotein scaffold most likely occurs during the conversion of III to IV. Possibly, this effect is mediated by coordination of the ylide to the heme iron and/or by interaction of the ylide with the distal cavity of the protein. While Mb has naturally evolved to bind small ligands (i.e. O_2), the steric feasibility of the putative intermediate III is suggested by our previous finding that the distal heme pocket in Mb can accomodate rather bulky ligands and substrates.[8, 13] This process can be further facilitated by the ability of the distal histidine, His64 (Figure S2), to swing open upon ligand binding[14], thereby creating a larger cavity above the heme.

Another interesting point concerns the structure-reactivity data obtained with the different diazo compounds (Table 3). These studies showed that while relatively large and bulky groups within the ester group of the carbenoid moiety are well tolerated by the Mb(F43V,V68F) catalyst, α-substitutions are not. Since we previosly established that ethyl

α-diazo-propanoate (**2e**) is a viable carbene donor in Mb-catalyzed olefin cyclopropanation,[8a] it can be derived that α-substitutions negatively affect catalytic steps downstream of the formation of the heme-carbene complex during aldehyde olefination. Reasonably, the increased steric hindrance provided by the α-methyl group may disfavour attack of the PPh_3 to the heme-carbene (II→III, Scheme 2), thus preventing formation of the key phosphonium ylide intermediate.

In spite of the high TON supported by Mb(F43V,V68F), the aldehyde-to-olefin conversion in this reaction was surprisingly found to not exceed 50%. Increasing the α-diazo ester : aldehyde ratio did not improve the yield and resulted in a larger amount of the carbene dimerization product. Through control experiments, catalyst inhibition by action of the aldehyde, phosphine, or olefin product could be ruled out as a possible cause for this phenomenon. A reduction in TON was observed however upon addition of increasing amounts of phosphine oxide to the Mb(F43V,V68F)-catalyzed reaction (Figure S3). Overcoming the inhibitory effect exerted by the phosphine/arsine oxide could thus provide a way to further enhance the efficiency of this Mb-mediated transformation in the future.

In summary, our results show that engineered myoglobins can provide efficient and selective biocatalysts for the olefination of aldehydes under mild and neutral conditions. To our knowledge, this report represents the first example of a biocatalytic strategy for aldehyde olefination. Using the most promising Mb-based catalyst identified in this work, Mb(F43V,V68F), a variety of aryl aldehydes and alkyl α-diazo acetates could be converted to the corresponding olefin products with high catalytic efficiency (1,100–4,900 TON) and very good to excellent *E*-selectivity (94–99.9% *de*). The Mb-catalyzed aldehyde olefination reported here contributes to expand the growing number of synthetically valuable transformations accessible through catalysis with engineered and artificial metalloproteins.[5, 7–8, 13, 16]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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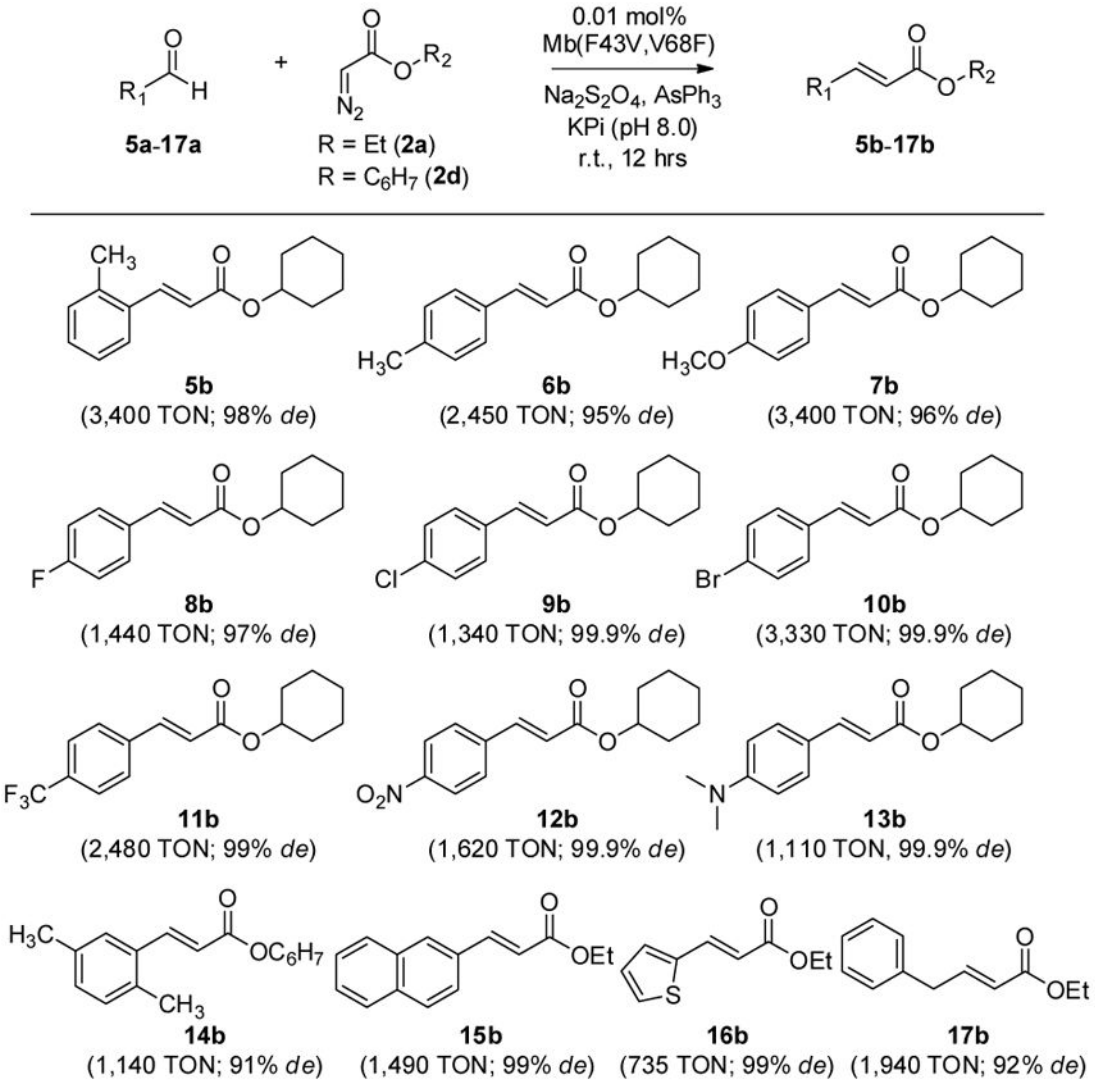
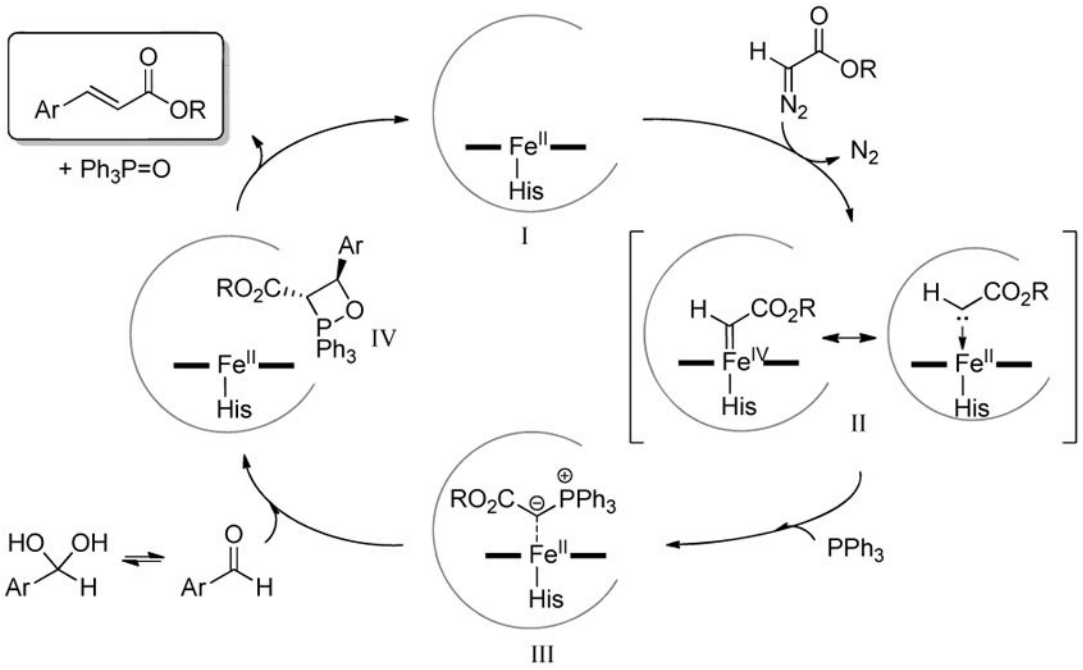


Scheme 1.

Substrate scope for Mb(H64V,V68A)-catalyzed aldehyde olefination. Reaction conditions: 10 mM aryl aldehyde, 1 μM Mb(F43V,V68F), 10 mM cyclohexyl α-diazo-acetate (**2d**), 10 mM $AsPh_3$, 10 mM $Na_2S_2O_4$.

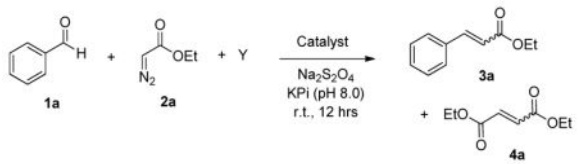


Scheme 2.

Proposed mechanism and catalytic steps for the myoglobin-catalyzed olefination of aryl aldehydes.

Table 1

Catalytic activity of hemin and wild-type sperm whale myoglobin (Mb) in the olefination of benzaldehyde with ethyl α-diazoacetate (EDA).[a]



Entry	Catalyst	Y	TON[b] (3a)	*de* (*E*)[c]	$TON_{(3a)}/TON_{(4a)}$
1	Hemin	PPh_3	22	64%	0.4
2	Hemin[d]	$AsPh_3$	4	91%	0.1
3	WT Mb	PPh_3	31	76%	2.8
4	WT Mb	$AsPh_3$	27	99.9%	0.7
5	WT Mb	$SbPh_3$	5	99.9%	0.4
6	WT Mb	$BiPh_3$	0	-	-

[a] Reactions were carried out under anaerobic conditions with 10 mM **1a**, 10 mM **2a**, 20 μM catalyst, 10 mM $Na_2S_2O_4$, and 10 mM **Y** for 12 hours at room temperature.

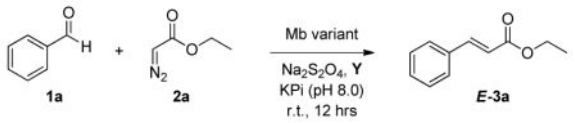
[b] = nmol olefin / nmol catalyst. Errors in reported values are within ± 10%.

[c] As determined by chiral gas chromatography.

[d] With hemin at 60 μM.

Table 2

Catalytic activity and selectivity of myoglobin variants in benzaldehyde olefination with EDA.[a]



Entry	Catalyst	Y	TON	*de (E)*	$TON_{(3a)}/TON_{(4a)}$
1	WT Mb	PPh_3	31	76%	2.8
		$AsPh_3$	27	99.9%	0.7
2	Mb(L29A)	PPh_3	16	79%	0.8
		$AsPh_3$	34	99.9%	0.5
3	Mb(F43V)	PPh_3	20	69%	1.2
		$AsPh_3$	35	99.9%	1.1
4	Mb(F43W)	PPh_3	30	68%	6.0
		$AsPh_3$	56	99.9%	2.7
5	Mb(H64V)	PPh_3	37	69%	1.7
		$AsPh_3$	35	99.9%	0.5
6	Mb(V68A)	PPh_3	13	69%	0.5
		$AsPh_3$	36	99.9%	1.1
7	Mb(V68F)[b]	PPh_3	28	64	0.7
		$AsPh_3$	82	94	1.9
8	Mb(L29A,H64V)	PPh_3	57	70%	1.4
		$AsPh_3$	50	80%	0.5
9	Mb(H64V,V68A)	PPh_3	7	73%	0.8
		$AsPh_3$	16	57%	0.2
10	Mb(F43V,V68F)	PPh_3	38	64%	2.9
		$AsPh_3$	92	99.9%	3.3
11	Mb(F43V,V68F)[c]	$AsPh_3$	1,170	99.9%	4.2

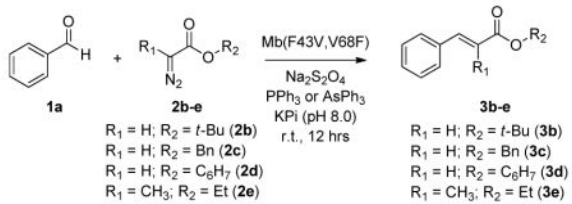
[a]Reaction conditions are the same as in Table 1.

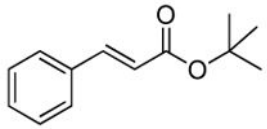
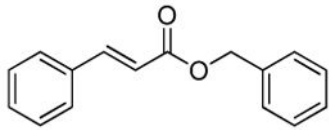
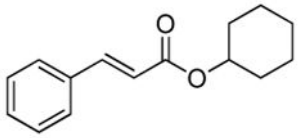
[b] With 5 μM catalyst (0.05 mol%).

[c] With 1 μM catalyst (0.01 mol%).

Table 3

Catalytic activity and selectivity of Mb(F43V,V68F) variants in benzaldehyde olefination with different α-diazo esters.[a]



Entry	Product	Y	TON	% *de*	% conv.[b]
1		PPh_3	160	79	32
2		$AsPh_3$	40	99.9	8
3[c]		$AsPh_3$	175	99.9	1.7
	3b				
3		PPh_3	185	83	37
4		$AsPh_3$	155	98	31
5[c]		$AsPh_3$	4,920	94	49
	3c				
5		PPh_3	155	79	31
6		$AsPh_3$	205	99.9	41
7[c]		$AsPh_3$	4,230	99.9	42
	3d				

[a]Reaction conditions as described in Table 1 using 20 μM catalyst (0.2 mol%).

[b]GC conversion.

[c]Using 1 μM catalyst (0.01 mol%).