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Aldehyde and Ketone Synthesis by P450-Catalyzed Oxidative Deamination of Alkyl Azides

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Heme-containing proteins have recently attracted increasing attention for their ability to promote synthetically valuable transformations not found in nature. Following the recent discovery that engineered variants of myoglobin can catalyze the direct conversion of organic azides into aldehydes, we investigated the azide oxidative deamination reactivity of a variety of hemoproteins featuring different heme coordination environments. Our studies show that although several heme-containing enzymes possess basal activity in this reaction, an engineered variant of the bacterial cytochrome P450 CYP102A1 constitutes a particularly efficient biocatalyst for promoting this transformation, and it exhibits a broad substrate scope along with high catalytic activity (up to 11 300 turnovers), excellent chemoselectivity, and enhanced reactivity toward secondary alkyl azides to yield ketones. Mechanistic studies and Michaelis–Menten analyses provided insight into the mechanism of the reaction and the impact of active-site mutations on the catalytic properties of the P450. Altogether, these studies demonstrate that engineered P450 variants represent promising biocatalysts for the synthesis of aryl aldehydes and ketones through the oxidative deamination of alkyl azides under mild reaction conditions.

Cytochrome P450s constitute a superfamily of iron-dependent, heme-containing oxygenases that play an important role in drug metabolism, biodegradation, and the biosynthesis of secondary metabolites.[1] These enzymes have received significant attention for their ability to hydroxylate aliphatic and aromatic C–H bonds, a challenging reaction to achieve by chemical means.[2] Furthermore, P450s are known to promote a variety of other oxidative transformations, including heteroatom dealkylation, carbon–carbon bond cleavage, rearrangement reactions, and Baeyer–Villiger reactions.[3] More recently, the scope of cytochrome P450s was extended to a number of important, "non-native" reactions useful for the construction of carbon–carbon,[4] carbon–nitrogen,[5] and nitrogen–sulfur[6] bonds.

Our group recently reported that the heme-containing protein myoglobin (Mb) constitutes a promising scaffold for promoting a variety of synthetically useful transformations mediated by metal–carbenoid and –nitrenoid species.[7] In particular, previous work showed that engineered variants of this hemoprotein can catalyze the oxidative deamination of organic azides to generate aldehydes.[8] In comparison to classical methods involving alcohol oxidation with toxic chromium-based reagents, this transformation provides a convenient approach to the synthesis of aldehydes starting from a non-oxygenated functional group and by using readily accessible alkyl azides. Furthermore, synthetic catalysts [e.g., $MoO_2(S_2CNEt_2)_2$] currently available to promote this reaction suffer from poor catalytic efficiency (20–200 turnovers) and require harsh reaction conditions (reflux in toluene/water mixture).[9] In the interest of comparing and contrasting the reactivities of hemoproteins featuring different heme coordination environments in the context of non-native reactions, we investigated and report herein the azide oxidative deamination reactivity of a panel of different heme-containing enzymes, including a catalase, a peroxidase, and wildtype and engineered variants of a bacterial cytochrome P450. These studies led to the identification of an engineered variant of CYP102A1 as a superior biocatalyst for the conversion of a broad range of aryl-substituted alkyl azides into the corresponding aldehydes and ketones. In addition, insight into the mechanism and catalytic properties of this enzyme was gained through a combination of kinetic studies and isotopic labeling experiments.

To investigate the scope of hemoprotein-catalyzed azide-to-aldehyde oxidation, we initially selected a panel of different heme-containing enzymes consisting of bovine catalase (Cat), horseradish peroxidase (HRP), and the bacterial cytochrome CYP102A1[10] (also known as $P450_{BM3}$).[11] The heme coordination environments among these enzymes are significantly different and, additionally, are different to that of the previously investigated Mb. In Cat and CYP102A1, the amino acid ligands involved in coordinating the heme iron are tyrosine and cysteine residues, respectively,[12] as opposed to histidine in Mb.[13] The heme group in HRP is also bound through a histidine residue,[14] but a strong H-bonding interaction between this proximal His and a neighboring aspartic acid residue confers considerable anionic character to the former, a structural feature that is not present in Mb. Finally, hemoglobin (Hb) was also considered to evaluate the effect of the tetrameric structure on the target reactivity as opposed to the monomeric structure of Mb.

As a model reaction, the conversion of benzyl azide (**1**) into benzaldehyde (**2a**) under anaerobic conditions in phosphate buffer (KPi, pH 7.0) and in the presence of sodium dithionite ($Na_2S_2O_4$) as a reductant (Figure 1a) was used to assess the relative efficiency of these hemoproteins in promoting azide oxidative deamination. Under these conditions, Cat, HRP, and Hb showed only low to moderate reactivity, which supported ap-

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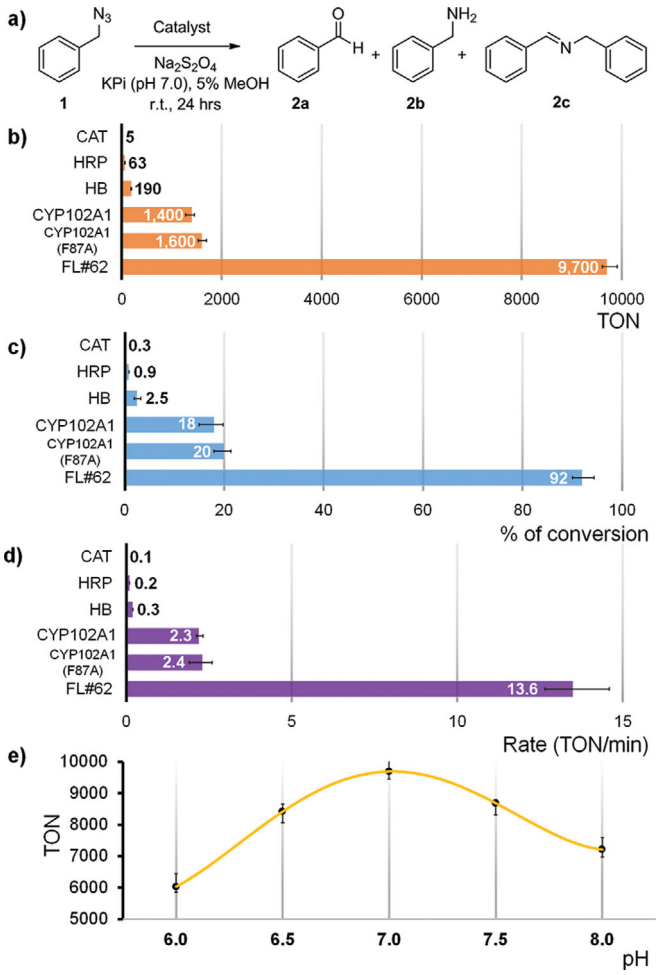


Figure 1. a) Catalytic activity of various hemoproteins in the oxidative deamination of benzyl azide (**1**) to benzaldehyde (**2a**). b,c) Reactions (400 μL) were conducted under anaerobic conditions with 10 mM BnN_3, 10 mM $Na_2S_2O_4$, and 1 μM protein for 24 h at room temperature. TON = nmol aldehyde/nmol catalyst. Product yield is based on conversion of **1** into **2a**, as determined by GC. d) Reaction rates as determined over the first 10 min of the reaction. e) TON versus pH plot for FL#62 reaction.

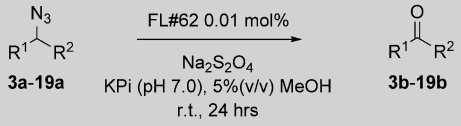
proximately 5, 63, and 195 turnovers (TONs), respectively (Figure 1b,c). These TON values are lower or comparable to that achieved with free hemin under similar reaction conditions ($\approx$100 TONs).[8] The poor performance of Hb was somewhat unexpected in light of the high reactivity of the structurally related Mb in this transformation (1650 TONs)[8] and it could stem from unfavorable allosteric effects in the case of the oligomeric protein. In contrast, wildtype CYP102A1 showed considerably higher azide oxidation activity, which supported 1400 catalytic turnovers for the formation of desired product **2a** and provided a product conversion ratio of 18% (Figure 1b,c) The reaction also produced small amounts of benzylamine (**2b**, 0.5%) and *N*-benzylbenzylimine (**2c**, $<$3%), as observed in the Mb-catalyzed reaction.[8] Formation of byproduct **2b** was ascribed to an unproductive pathway resulting from decomposition of the azide followed by reduction and protonation of the resulting nitrene-heme intermediate, whereas **2c** originates from condensation of **2b** with the aldehyde product.[8] Importantly, these initial results clearly evidenced the impact of the heme coordination environment on the reactivity of the different hemoproteins toward organic azides, thereby expanding previous findings from our group.[7e] Indeed, whereas HRP was previously found to promote the intramolecular C–H amination of arylsulfonyl azides with comparable or higher activity than P450s or Mb,[5a,b,7e] this enzyme showed significantly reduced activity toward azide-to-aldehyde conversion compared to the latter hemoproteins. Conversely, the data in Figure 1b,c suggested that, despite the inherent differences between their active sites, the thiolate-bound heme in P450 is equally efficient as the histidinyl-bound heme cofactor in Mb in promoting the oxidative deamination of alkyl azide **1** (1400 TONs vs. 1650 TONs for wildtype Mb[8]).

Given the promising activity of wildtype CYP102A1, we next extended our investigations to two engineered variants of this enzyme, namely, CYP102A1(F87A) and FL#62. The former variant was chosen because of the well-documented ability of the F87A mutation to expand the substrate profile of CYP102A1 toward non-native substrates in the context of monooxygenation reactions.[11,15] In addition to possessing a broad substrate profile,[16] the FL#62 variant, which carries 6 active-site mutations and 10 additional mutations within its heme domain, was chosen because of its high reactivity in the context of the C–H amination of azide-based reagents.[5a,b] As shown by the data summarized in Figure 1, CYP102A1(F87A) showed only slightly improved TON values for the conversion of benzyl azide (**1**) into benzaldehyde (**2a**) relative to the wildtype P450 (1600 vs. 1400 turnovers). In contrast, FL#62 was found to catalyze this transformation with considerably higher efficiency (Figure 1b,c), supporting 9700 catalytic turnovers and providing nearly quantitative product conversion (92%) at a catalyst loading of merely 0.001 mol%. At lower catalyst loadings (0.0005 mol%), a TON value as high as 11300 was measured for this enzyme. Notably, the FL#62 reaction was found to proceed also with excellent chemoselectivity, which led to the clean formation of **2a** without producing detectable amounts of byproduct **2b** or **2c**. Altogether, these properties make FL#62 not only a considerably more efficient catalytic system for azide-to-aldehyde conversion than synthetic Mo-based catalysts (50–99% conversions by using 10 mol% at 100 °C in water/toluene mixture)[9a] but also a superior biocatalyst to the best-engineered Mb variant [Mb(H64V,V68A)] identified in the course of our previous studies.[8]

Further characterization studies revealed a noticeable effect of pH on FL#62 azide oxidative deamination activity, and maximal TON values were achieved around neutral pH (Figure 1e). A similar pH dependence was observed for the Mb-catalyzed reaction.[8] From time-course experiments, the FL#62-catalyzed conversion of **1** into **2b** was determined to proceed with a rate of approximately 14 turnovers per minute over the first 10 min (Figure 1d). This rate is lower than that observed for the Mb-based catalysts (250 min^{-1}),[8] but the FL#62-catalyzed reaction proceeded with higher selectivity (i.e., no **2b**/**2c** formation) in addition to the higher TON value. The initial product formation rates for FL#62 and the other hemoproteins investigated also correlated well with the TON values observed in the corresponding reactions (Figure 1b,d).

Next, the substrate scope of the FL#62 biocatalyst was investigated by using a wide panel of different organic azides. As shown in Table 1, the majority of these compounds were readily oxidized to the corresponding aldehydes in good to excellent yields (49–99%), as determined by GC. In these reactions, P450 was determined to support from 4650 to 9660 TONs. In addition, the reaction proceeded with high selectivity (>90–95%), in particular for the alkyl azides most efficiently processed by the enzyme. The viable substrates included variously mono- and disubstituted benzyl azide derivatives (i.e., **3a**, **4a**, **6a**, **8a**, **10a**, and **11a**), (hetero)aryl-substituted primary alkyl azides (i.e., **17a** and **18a**), and secondary alkyl azides (i.e., **15a** and **16a**), which supported the broad substrate scope of the FL#62 catalyst. Modest (20–30%) to low (<20%) product conversions were observed instead with benzyl azide derivatives that carried one or two substituents at the *ortho* position(s) (i.e., **7b**, **9b**, and **13b**). These results suggested a negative effect on the enzymatic transformation of increased steric hindrance in proximity to the azido group. Whereas the conversion yields of these P450-catalyzed reactions were consistently higher than those achieved with engineered myoglobins,[8] particularly striking is the considerably higher efficiency of the P450 catalyst in the synthesis of ketones **15b** and **16b** from secondary alkyl azides **15a** and **16a**, respectively. Indeed, whereas this reaction proceeded inefficiently in the presence of the Mb catalyst (280–400 TONs, <2% conversion),[8] it was effectively catalyzed by the engineered P450 (4650–7470 TONs), which resulted in much improved product conversions (49–76%).

Table 1. Substrate scope for the FL#62-catalyzed oxidative deamination of alkyl azides.[a]



Product	R^1	R^2	Conversion [%]	Selectivity [%]	TON
3b	H	4-MeC_6H_4	58	94	6000
4b	H	4-MeOC_6H_4	99	99	9440
5b	H	4-$O_2NC_6H_4$	25	84	2670
6b	H	4-$F_3CC_6H_4$	69	93	6820
7b	H	2-MeC_6H_4	22	79	2000
8b	H	2-FC_6H_4	96	98	9660
9b	H	2-$O_2NC_6H_4$	1	18	60
10b	H	3-$O_2NC_6H_4$	67	94	6600
11b	H	2,4-$F_2C_6H_3$	51	90	5850
12b	H	3,5-$Me_2C_6H_3$	36	88	3540
13b	H	2,6-$Cl_2C_6H_3$	7	72	720
14b	Me	Ph	5	51	490
15b	CF_3	Ph	76	98	7470
16b	CO_2Me	Ph	49	91	4650
17b	H	1-naphthyl	70	94	7480
18b	H	2-thienyl	97	98	9500
19b	H	PhCH=CH	19	85	1970

[a] Reaction conditions: azide (10 mM), FL#62 (1 μM), $Na_2S_2O_4$ (10 mM).

To further evaluate the synthetic utility of FL#62 in the context of this reaction, a larger scale reaction with benzyl azide (**1**; 50 mg, 0.38 mmol) was performed in the presence of 0.05 mol% FL#62 in phosphate buffer (pH 7.0) at room temperature. After simple extraction and purification steps, the desired benzaldehyde product **2a** was isolated in 87% yield (35 mg, 0.33 mmol), which thus supported the scalability of this biocatalytic transformation.

With respect to the mechanism, we previously proposed that this reaction involves heme-catalyzed decomposition of the heme-bound alkyl azide to give an imido–iron(IV) intermediate. The latter could then undergo tautomerization to an imine–iron(II) complex, followed by hydrolysis of the imine (in either free or heme-bound form) to yield the aldehyde product (Figure 2). Experimental evidence in support of the imine tautomerization step included the observation of a significant kinetic isotope effect (KIE) upon H→D substitution at the level of the α carbon atom in the azide substrate.[8] Interestingly, a related imine intermediate was recently invoked in the azide-to-nitrile conversion catalyzed by non-heme iron-dependent dioxygenases.[17] Furthermore, ^{18}O-labeling experiments indicated that the oxygen atom in the aldehyde product derives from a hydrolytic process.[8] In light of the higher catalytic activity (i.e., TON) and slower rate of the P450-catalyzed reaction versus the Mb-catalyzed one, we thus wondered whether these biocatalytic transformations shared an analogous mechanism or not. To this end, we measured the KIE for the FL#62-catalyzed oxidative deamination of benzyl azide (**1**) from competition experiments in the presence of an equimolar concentration of the deuterated analogue, [D_2]-**1**. GC–MS analysis of this reaction (Figure S3a, Supporting Information) yielded a KIE value (k_H/k_D) of 1.9±0.2 at 22 °C, which is comparable to that observed in the presence of Mb(H64V,V68A) as the catalyst (k_H/k_D=1.7±0.3).[8] In addition, the FL#62-catalyzed conversion of benzyl azide (**1**) in the presence of ^{18}O-labeled water resulted in the formation of ^{18}O-labeled aldehyde product **2a**(^{18}O) (Figure S3b), as observed for the Mb-catalyzed reaction. Altogether, these results hint at important mechanistic similarities between the two systems, although differences between them are also apparent. Indeed, the low formation of the amine by-product in the FL#62 reactions across all the tested azide sub-

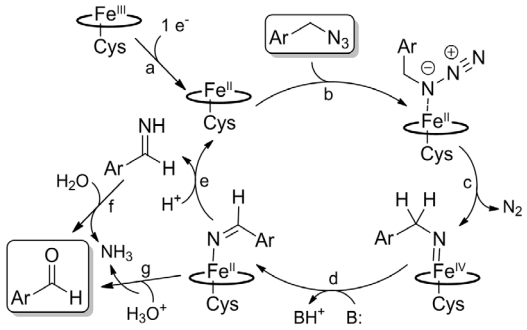


Figure 2. Proposed mechanism and catalytic steps for the P450-catalyzed oxidation of alkyl azides to aldehydes.

strates suggests that the unproductive pathway leading to this reduction is more disfavored in the case of the P450 system. In addition, the significantly higher (10- to 15-fold) conversions and TON values measured for the FL#62-catalyzed formation of **15b** and **16b** versus **14b** (Table 1) suggest that the P450 catalyst is significantly more sensitive to an increase in the acidity of the α protons than the Mb catalyst (three- to fourfold higher TON for **15b** and **16b**, respectively, relative to the TON for **14b**).[8]

Kinetic experiments with wildtype CYP102A1, CYP102A1(F87A), and FL#62 were performed to gain further insight into the catalytic properties of these enzymes. In the reaction with model substrate **1**, all of these P450s were found to follow Michaelis–Menten kinetics (Figure 3). Similar kinetic parameters (Michaelis constant, K_M; turnover number, k_{cat}) were

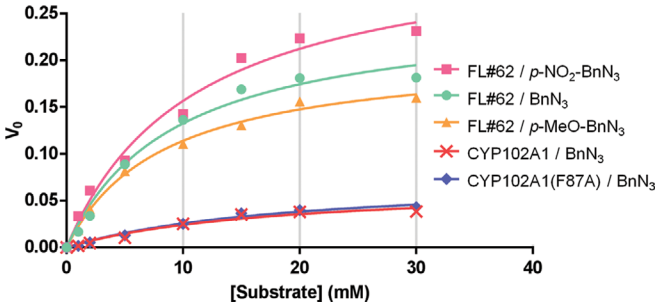


Figure 3. Overlay plot of initial velocity (V_0) versus substrate concentration for the reactions of CYP102A1 and its variants with selected alkyl azide substrates. The corresponding kinetic parameters (k_{cat}, K_M, k_{cat}/K_M) are reported in Table 2.

determined for the parent enzyme and its single mutant variant, CYP102A1(F87A), which is consistent with their similar performance in terms of TON and product conversion (Table 1). Conversely, the superior catalytic performance of FL#62 is reflected by the eight fold higher catalytic efficiency ($k_{cat}/K_M = 4.6\times10^2$ M s^{-1}) compared to that of the parent enzyme and F87A variant, which derives as a result of a twofold lower K_M value and a fourfold faster turnover number, k_{cat} (Table 2). Whereas the catalytic efficiency of FL#62 in this non-native reaction is significantly lower than that of CYP102A1 for the hydroxylation of fatty acids ($k_{cat}/K_M \approx 10^5$ M^{-1} s^{-1})[18], it falls in the same range as those exhibited by P450s involved in drug and hormone metabolism (e.g., P4501B1/retinol, $k_{cat}/K_M \approx 7\times10^3$ M^{-1} s^{-1}).[19] For all the P450s, incubation with benzyl azide resulted in a small (<5–10%) but detectable heme spin shift (Figure S4), which is indicative of the displacement of the axial water ligand on the heme by the bound substrate. Titration experiments revealed that FL#62 has a higher binding affinity for benzyl azide than wildtype CYP102A1 and CYP102A1(F87A) (dissociation constant, K_D, of 68 μM vs. 93 and 84 μM, respectively; Table 2; Figure S4).

Another interesting result emerging from our substrate scope investigations with FL#62 is the noticeable difference between the TON values associated with the synthesis of electron-deficient benzyl aldehydes (e.g., **5b**; 2670 TONs) and those associated with electron-rich ones (e.g., **4b**; 9440). To elucidate the basis of this trend, we extended the substrate binding experiments and Michaelis–Menten analyses to the FL#62-catalyzed oxidative deamination of *p*-nitrobenzyl azide (**5a**) and *p*-methoxybenzyl azide (**4a**). Whereas *p*-nitrobenzyl azide had weaker affinity for FL#62 than benzyl azide and *p*-methoxybenzyl azide (Table 2), no significant differences were found among the kinetic parameters (K_M, k_{cat}) and catalytic efficiency (k_{cat}/K_M) of the enzyme for the transformation of these substrates, which suggested that other factors must be at the basis of the less efficient conversion of **5a** compared to **4a** and **1**.

To further examine this aspect, we performed product inhibition experiments in which reaction mixtures containing FL#62 and **1**, **4a**, or **5a** as the substrate were spiked with increasing concentrations (0–10 mM) of the corresponding aldehyde products. Interestingly, the addition of *para*-nitrobenzaldehyde (**5b**) significantly reduced the conversion of **5a** (Figure 4a), which evidenced the occurrence of product inhibition. In contrast, no product inhibition was observed within the concentration range tested for both benzyl azide (**1**) (Figure S5) and *para*-methoxybenzyl azide (**4b**) (Figure 4b), thus explaining the greater yields observed in the presence of these substrates (Table 1).

In conclusion, our results demonstrate that cytochrome P450s are promising catalysts for the synthesis of aromatic aldehydes through the oxidative deamination of alkyl azides. In particular, we established that the engineered CYP102A1 variant FL#62 represents a superior biocatalyst for this transformation relative to other heme-containing enzymes and the previously reported Mb catalysts. In addition to excellent chemose-

Table 2. Substrate binding affinity and Michaelis–Menten parameters corresponding to CYP102A1, CYP102A1(F87A), FL#62, and selected alkyl azide substrates.[a]

Enzyme	Substrate	K_D [μM]	K_M [M]	k_{cat} [min^{-1}]	k_{cat}/K_M [M^{-1} s^{-1}]
CYP102A1	BnN_3 (**1**)	93 (±9)	1.8×10^{-2} (±0.7)	66 (±13)	61 (±27)
CYP102A1(F87A)	BnN_3 (**1**)	84 (±13)	1.9×10^{-2} (±0.5)	75 (±11)	66 (±20)
FL#62	BnN_3 (**1**)	68 (±10)	9.2×10^{-3} (±1.9)	255 (±20)	4.6×10^2 (±1.0)
FL#62	4-$MeOC_6H_4CH_2N_3$ (**4a**)	107 (±19)	8.3×10^{-3} (±1.0)	209 (±9)	4.2×10^2 (±0.5)
FL#62	4-$O_2NC_6H_4CH_2N_3$ (**5a**)	259 (±36)	1.1×10^{-2} (±0.2)	329 (±29)	5.0×10^2 (±1.0)

[a] Equilibrium dissociation constants (K_D) were calculated from substrate-induced heme spin shift experiments (Figure S4). The kinetic parameters (k_{cat}, K_M, k_{cat}/K_M) were calculated by nonlinear fitting of the V_0 vs. concentration curves (Figure 3) to the Michaelis–Menten equation.

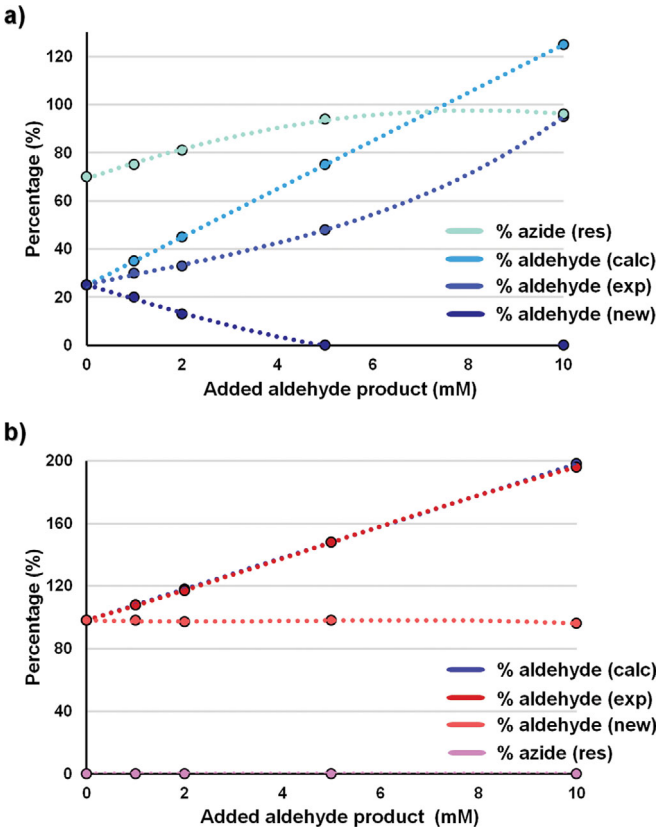


Figure 4. Product inhibition experiments for a) the reaction of FL#62 and *p*-nitrobenzyl azide (**5a**) in the presence of added *p*-nitrobenzaldehyde (**5b**) and b) the reaction of FL#62 and *p*-methoxybenzyl azide (**4a**) in the presence of added *p*-methoxybenzaldehyde (**4b**). See the caption of Figure S5 for further details.

lectivity and high catalytic activity (up to 11 300 TONs) across a broad range of alkyl azides, the enhanced reactivity of this P450 enabled extension of this transformation to the synthesis of ketones from secondary azides. These studies contributed valuable insight into the differential reactivities of the hemoproteins and engineered variants thereof in the context of non-native reactions, which is expected to aid the future development of biocatalysts for a growing number of synthetically useful transformations not found in nature.

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[1] a) F. P. Guengerich, *Nat. Rev. Drug Discovery* **2002**, *1*, 359–366; b) S. Kumar, *Expert Opin. Drug Metab. Toxicol.* **2010**, *6*, 115–131; c) I. G. Denisov, T. M. Makris, S. G. Sligar, I. Schlichting, *Chem. Rev.* **2005**, *105*, 2253–2277; d) T. L. Poulos, *Biochem. Biophys. Res. Commun.* **2005**, *338*, 337–345.

[2] a) R. Fasan, *ACS Catal.* **2012**, *2*, 647–666; b) J. C. Lewis, P. S. Coelho, F. H. Arnold, *Chem. Soc. Rev.* **2011**, *40*, 2003–2021; c) A. Chefson, K. Auclair, *Mol. BioSyst.* **2006**, *2*, 462–469; d) V. B. Urlacher, M. Girhard, *Trends Biotechnol.* **2012**, *30*, 26–36; e) E. M. J. Gillam, M. A. Hayes, *Curr. Top. Med. Chem.* **2013**, *13*, 2254–2280; f) G. Grogan, *Curr. Opin. Chem. Biol.* **2011**, *15*, 241–248.

[3] a) E. M. Isin, F. P. Guengerich, *Biochim. Biophys. Acta Gen. Subj.* **2007**, *1770*, 314–329; b) P. R. Ortiz de Montellano, S. D. Nelson, *Arch. Biochem. Biophys.* **2011**, *507*, 95–110; c) F. P. Guengerich, A. W. Munro, *J. Biol. Chem.* **2013**, *288*, 17065–17073.

[4] a) P. S. Coelho, E. M. Brustad, A. Kannan, F. H. Arnold, *Science* **2013**, *339*, 307–310; b) Z. J. Wang, H. Renata, N. E. Peck, C. C. Farwell, P. S. Coelho, F. H. Arnold, *Angew. Chem. Int. Ed.* **2014**, *53*, 6810–6813; *Angew. Chem.* **2014**, *126*, 6928–6931.

[5] a) R. Singh, M. Bordeaux, R. Fasan, *ACS Catal.* **2014**, *4*, 546–552; b) R. Singh, J. N. Kolev, P. A. Sutera, R. Fasan, *ACS Catal.* **2015**, *5*, 1685–1691; c) J. A. McIntosh, P. S. Coelho, C. C. Farwell, Z. J. Wang, J. C. Lewis, T. R. Brown, F. H. Arnold, *Angew. Chem. Int. Ed.* **2013**, *52*, 9309–9312; *Angew. Chem.* **2013**, *125*, 9479–9482; d) T. K. Hyster, C. C. Farwell, A. R. Buller, J. A. McIntosh, F. H. Arnold, *J. Am. Chem. Soc.* **2014**, *136*, 15505–15508.

[6] C. C. Farwell, J. A. McIntosh, T. K. Hyster, Z. J. Wang, F. H. Arnold, *J. Am. Chem. Soc.* **2014**, *136*, 8766–8771.

[7] a) M. Bordeaux, V. Tyagi, R. Fasan, *Angew. Chem. Int. Ed.* **2015**, *54*, 1744–1748; *Angew. Chem.* **2015**, *127*, 1764–1768; b) V. Tyagi, R. B. Bonn, R. Fasan, *Chem. Sci.* **2015**, *6*, 2488–2494; c) G. Sreenilayam, R. Fasan, *Chem. Commun.* **2015**, *51*, 1532–1534; d) V. Tyagi, R. Fasan, *Angew. Chem. Int. Ed.* **2016**, *55*, 2512–2516; *Angew. Chem.* **2016**, *128*, 2558–2562; e) M. Bordeaux, R. Singh, R. Fasan, *Bioorg. Med. Chem.* **2014**, *22*, 5697–5704.

[8] S. Giovani, R. Singh, R. Fasan, *Chem. Sci.* **2016**, *7*, 234–239.

[9] a) M. Maddani, K. R. Prabhu, *Tetrahedron Lett.* **2008**, *49*, 4526–4530; b) H.-P. Zhang, Y.-Z. Dai, L.-M. Tao, *J. Chem. Res.* **2011**, *35*, 720–722.

[10] L. O. Narhi, A. J. Fulco, *J. Biol. Chem.* **1986**, *261*, 7160–7169.

[11] C. J. Whitehouse, S. G. Bell, L. L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218–1260.

[12] a) I. Fita, M. G. Rossmann, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 1604–1608; b) H. Li, T. L. Poulos, *Nat. Struct. Biol.* **1997**, *4*, 140–146.

[13] F. Yang, G. N. Phillips, Jr., *J. Mol. Biol.* **1996**, *256*, 762–774.

[14] G. I. Berglund, G. H. Carlsson, A. T. Smith, H. Szoke, A. Henriksen, J. Hajdu, *Nature* **2002**, *417*, 463–468.

[15] a) C. F. Oliver, S. Modi, M. J. Sutcliffe, W. U. Primrose, L. Y. Lian, G. C. Roberts, *Biochemistry* **1997**, *36*, 1567–1572; b) U. Schwaneberg, C. Schmidt-Dannert, J. Schmitt, R. D. Schmid, *Anal. Biochem.* **1999**, *269*, 359–366; c) A. B. Carmichael, L. L. Wong, *Eur. J. Biochem.* **2001**, *268*, 3117–3125; d) Q. S. Li, J. Ogawa, S. Shimizu, *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1258–1261; e) P. C. Cirino, F. H. Arnold, *Angew. Chem. Int. Ed.* **2003**, *42*, 3299–3301; *Angew. Chem.* **2003**, *115*, 3421–3423; f) J. C. Lewis, S. M. Mantovani, Y. Fu, C. D. Snow, R. S. Komor, C. H. Wong, F. H. Arnold, *ChemBioChem* **2010**, *11*, 2502–2505; g) E. Weber, A. Seifert, M. Antonovici, C. Geinitz, J. Pleiss, V. B. Urlacher, *Chem. Commun.* **2011**, *47*, 944–946; h) J. N. Kolev, J. M. Zaengle, R. Ravikumar, R. Fasan, *ChemBioChem* **2014**, *15*, 1001–1010.

[16] a) K. Zhang, S. El Damaty, R. Fasan, *J. Am. Chem. Soc.* **2011**, *133*, 3242–3245; b) K. D. Zhang, B. M. Shafer, M. D. Demars, H. A. Stern, R. Fasan, *J. Am. Chem. Soc.* **2012**, *134*, 18695–18704; c) J. N. Kolev, K. M. O'Dwyer, C. T. Jordan, R. Fasan, *ACS Chem. Biol.* **2014**, *9*, 164–173.

[17] M. A. Vila, M. Pazos, C. Iglesias, N. Veiga, G. Seoane, I. Carrera, *ChemBioChem* **2016**, *17*, 291–295.

[18] M. A. Noble, C. S. Miles, S. K. Chapman, D. A. Lysek, A. C. Mackay, G. A. Reid, R. P. Hanzlik, A. W. Munro, *Biochem. J.* **1999**, *339*, 371–379.

[19] D. Choudhary, I. Jansson, I. Stoilov, M. Sarfarazi, J. B. Schenkman, *Drug Metab. Dispos.* **2004**, *32*, 840–847.

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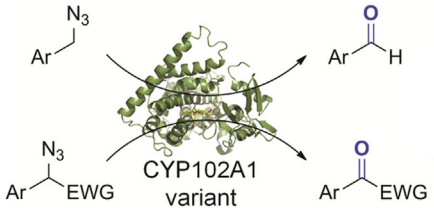
Aldehyde and Ketone Synthesis by P450-Catalyzed Oxidative Deamination of Alkyl Azides



- Up to 99% conversion
- Up to 11,300 turnovers
- k_{cat}/K_M = 4.6 x 10^2 M s^{-1} (benzyl azide)

Move azide: An engineered variant of CYP102A1 efficiently catalyzes the oxidative deamination of primary alkyl azides to yield aldehydes and exhibits a broad substrate scope along with high catalytic activity and excellent chemoselectivity. The enhanced reactivity of this enzyme also enables the conversion of selected secondary alkyl azides into ketones.