Myoglobin-Catalyzed Olefination of Aldehydes

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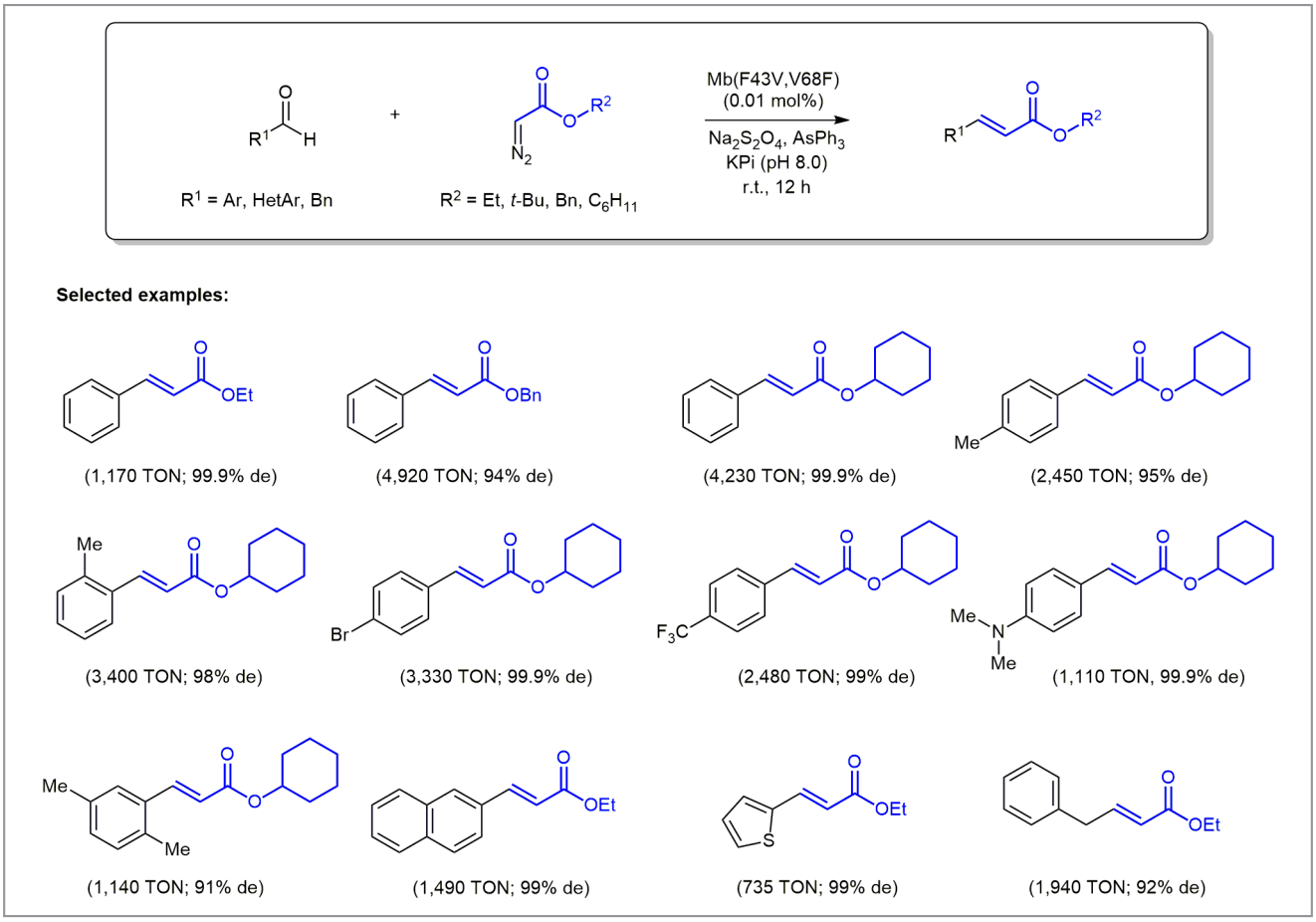
Biocatalytic processes are widely used by pharmaceutical companies for synthesizing small-molecule drugs and intermediates. Biocatalysis is currently of enormous interest both in academia and industry for its capacity to produce a wide range of chemicals under more environmentally and economically sustainable conditions. Recently, Professor Rudi Fasan's group at the University of Rochester (USA) reported the first example of a biocatalytic aldehyde olefination. Essentially, the authors demonstrated that engineered active-site variants of myoglobin constitute efficient catalysts for the conversion of aryl aldehydes into olefins in the presence of α-diazo esters and triphenylphosphine or triphenylarsine. This reaction is equivalent to the venerable and widely utilized Wittig reaction[1,2] but it proceeds under neutral instead of basic conditions, thus eliminating incompatibility problems with base-sensitive functional groups in the reactants. Professor Fasan explained: "This transformation was previously known to be catalyzed by synthetic transition-metal catalysts,[3–9] but an enzymatic counterpart was not available prior to our work. Most importantly, we developed a myoglobin-based catalyst, Mb(F43V,V68F), that can promote this transformation with exquisite diastereoselectivity (95–99.9% $de_{(trans)}$) while supporting one to two orders of magnitude higher catalytic turnovers (1,100–4,900 TON) than state-of-the-art transition-metal-based catalysts (Scheme 1)." In addition, the myoglobin-catalyzed reaction proceeds in aqueous solvent and at



Scheme 1 Substrate scope of Mb(F43V,V868F)-catalyzed olefination of aldehydes

room temperature, while previously reported methods require aromatic solvents and elevated temperatures. Notably, Mb(F43V,V68F) also exhibits a remarkably broad substrate scope and could be readily applied for the transformation of a variety of aldehyde substrates, including electron-rich and electron-deficient benzaldehyde derivatives, heteroaromatic aldehydes, and benzylic aldehydes (Scheme 1).

"My group has been interested in exploring the synthetic potential of myoglobin and other heme-containing proteins as catalytic platforms for promoting nitrene- and carbene-transfer reactions,"[10–12] said Professor Fasan. Having previously established that engineered variants of myoglobin can catalyze carbene-mediated reactions such as olefin cyclopropanation, N–H and S–H insertions with high efficiency and selectivity, a key insight that opened the way to the present work derived from the mechanistic studies on myoglobin-catalyzed S–H functionalization of mercaptans performed by the Fasan group, which supported the possibility of forming a myoglobin-bound sulfonium ylide catalytic intermediate.[12] "We reasoned that if a phosphonium ylide could be generated upon attack of a phosphine to the electrophilic heme-carbene complex with the active site of the protein, such intermediate could then engage an aldehyde substrate in a Wittig-type olefination reaction," said Professor Fasan. He continued: "At the planning stage, we were uncertain about whether a bulky tertiary phosphine such as PPh_3 could access the active site of the protein to undergo the envisioned catalytic process. Some initial experimentation, however, showed us the feasibility of the strategy and, along with it, the plasticity of the myoglobin scaffold." Further optimization studies indicated the superiority of triphenylarsine compared to triphenylphosphine to allow for the olefination reaction to proceed with high *E*-selectivity. The reasons for this effect are not entirely clear and the group hopes that computational studies will soon provide insights into this matter.

Professor Fasan said: "Next, we screened a panel of myoglobin variants with modified active sites in order to identify one that provided the optimal combination of high catalytic activity with high chemo- and stereoselectivity. Mb(F43V,V68F) (Figure 1) emerged as the most promising catalyst in this initial study, but it is clear that there is room for improvement." Among the objectives for the group is the development of myoglobin-based catalysts that can provide higher product conversions, for example by engineering them to be less sensitive to product inhibition. According to Professor Fasan, another challenge will be to develop myoglobin-based catalysts that can offer high levels of catalytic activity and selectivity toward aldehyde olefination in the presence of phosphine-based reagents instead of the considerably more expensive arsenic-based counterparts. "Among others, an attractive feature of the myoglobin system is that whereas it presents a well-defined active site (Figure 1), there are countless ways in which such an active site can be reshaped by mutagenesis in order to implement and fine-tune these catalytic properties," explained Professor Fasan, continuing: "Furthermore, libraries of these genetically encoded biocatalysts can be readily produced and screened in a high-throughput manner to facilitate catalyst optimization efforts. Last but not least, we are gathering accumulating evidence from this and our previous studies that, unlike most enzymes, these myoglobin-based catalysts exhibit a remarkably broad substrate profile and predictable reactivity. So, they seem to combine the best of the two worlds, that is the exquisite selectivity characteristic of biological catalysts with the predictable reactivity and broad substrate scope typical of synthetic catalysts."

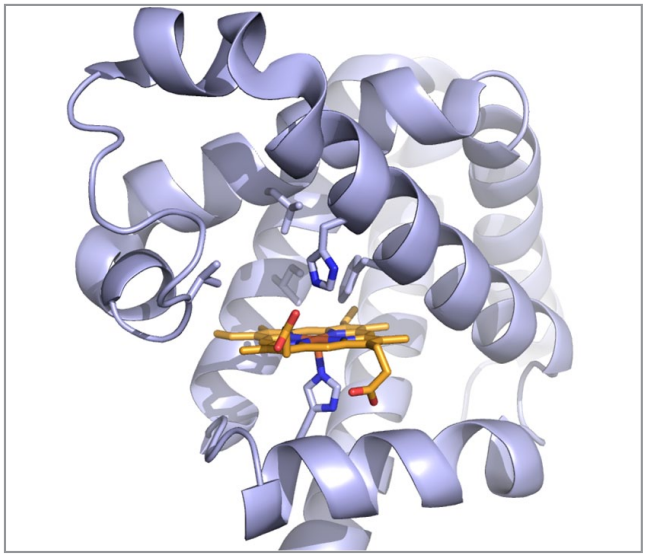
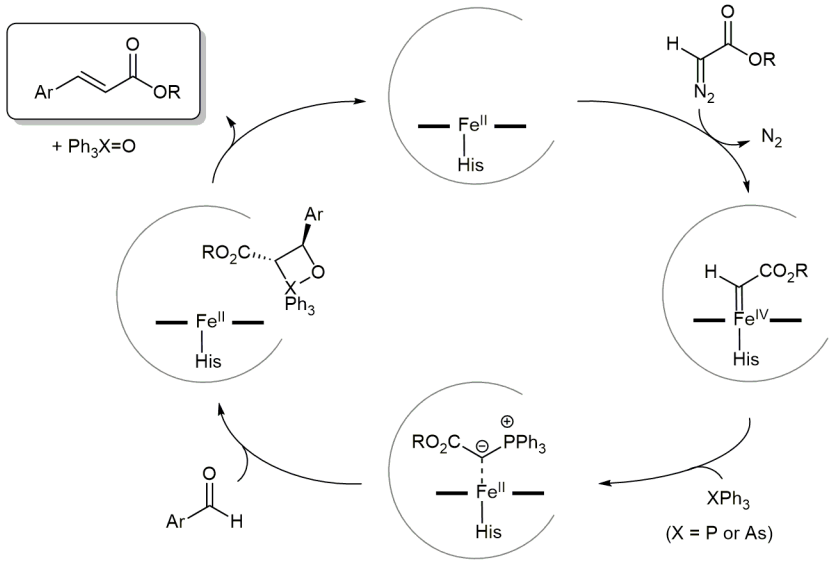


Figure 1 Model of Mb(F43V,V868F) catalyst highlighting the heme cofactor and active site residues (stick models)

In terms of mechanism, the authors of this study suspect that the reaction involves the formation of an eletrophilic heme-bound carbenoid intermediate that reacts with the tertiary phosphine/arsine to give rise to a phosphonium/arsonium ylide. The latter then reacts with the aldehyde to form an oxaphosphetane/oxarsetane intermediate which rearranges to yield the olefination product along with phosphine/arsine oxide as the byproduct (Scheme 2). "An intriguing, open question concerns how the protein scaffold transfers chirality onto the catalytic steps and intermediates to drive the reaction with an excellent degree of stereoselectivity," said Professor



Scheme 2 Plausible mechanism for myoglobin-catalyzed aldehyde olefination reaction

Fasan. "Our experiments suggest that this must occur during the formation of the oxaphosphetane/oxarsetane intermediate but the molecular mechanisms behind this process await further elucidation."

Professor Fasan revealed that this project was single-handedly executed by a very talented and dedicated postdoctoral fellow, Dr. Vikas Tyagi, who has been complementing his background in diversity-oriented synthesis with experience in biocatalysis, mechanistic enzymology, and chemoenzymatic synthesis.

"Biocatalysis is covering an increasingly important role in the manufacturing of fine chemicals, advanced pharmaceutical intermediates, and pharmaceuticals. However, the scope of biocatalysis in this field is currently limited to chemical transformations carried out by natural enzymes," said Professor Fasan, who concluded: "With this and other contributions from our group, we hope to change this paradigm by making available new classes of biocatalysts for promoting synthetically valuable carbon–carbon and carbon–heteroatom bond forming reactions not found in nature."

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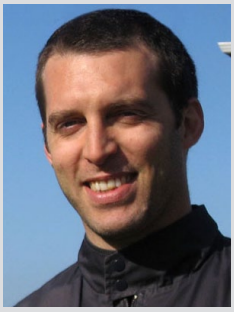
About the authors



Prof. R. Fasan

Rudi Fasan was born in Italy and received his B.Sc. from the University of Padua (Italy) in 1999. He obtained his Ph.D. in 2005 from the University of Zurich (Switzerland) working on beta-hairpin protein epitope mimetics under the supervision of Professor John Robinson. He then joined the Professor Frances Arnold's group at the California Institute of Technology (USA) as a Swiss National Science Foundation postdoctoral fellow, working on the directed evolution of P450 enzymes for alkane oxidation. Rudi began his independent career in the Department of Chemistry at the University of Rochester (USA) in 2008 and was promoted to the rank of Associate Professor in 2014. His laboratory focuses on the synthesis and investigation of peptide-based macrocycles as inhibitors of protein–protein interactions and on the design, development, and application of metalloprotein catalysts for C(sp^3)–H functionalization and asymmetric carbon–carbon and carbon–heteroatom bond formation.

Dr. V. Tyagi

Vikas Tyagi was born and raised in Uttar Pradesh (India). He obtained his M.Sc. in 2007 from C.C.S. University (Meerut, India). In 2013, he received his Ph.D. in chemistry from Central Drug Research Institute at Jawaharlal Nehru University (Delhi, India) under the supervision of Dr. Prem M. S. Chauhan, where he worked on diversity-oriented synthesis of biologically active N-heterocyclic compounds. He joined the group of Professor Rudi Fasan at the University of Rochester (USA) as a postdoctoral fellow in December of 2013. His postdoctoral research has focused on the development and investigation of engineered myoglobin catalysts for promoting 'non-native' carbene-mediated organic transformations.

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