catalysts

MDPI

*Editorial*

# Editorial: Special Issue on "Flavin Monooxygenases"

**Gianfranco Gilardi and Sheila J. Sadeghi ***

Department of Life Sciences and Systems Biology, University of Torino, 10123 Torino, Italy; gianfranco.gilardi@unito.it
* Correspondence: sheila.sadeghi@unito.it

Flavin-containing monooxygenase are a large family of enzymes involved in an array of different reactions by activating molecular oxygen and transferring one atom of oxygen to their substrates. They are present in all kingdoms of life from bacteria to humans. In particular, the single-component enzymes of class B, including flavin-containing monooxygenases (FMOs) and Baeyer-Villiger monooxygenases (BVMOs) have recently been under intense research due to the benefits of "Green Chemistry" and enzymatic oxyfunctionalisation under more environmentally friendly conditions utilising fewer toxic reagents and ambient temperatures. These enzymes are important oxidoreductases with immense potential for development as biocatalysts within the chemical, biotechnological and pharmaceutical industries. More and more knowledge is being generated about their catalytic cycle and on their engineered forms.

This special issue includes three different review papers starting from the endogenous roles of mammalian FMOs [1]. The authors review the roles of the five human FMO isoforms involved in the metabolism of endogenous substrates and in physiological processes. Tyramine, phenethylamine, trimethylamine, cysteamine, methionine, lipoic acid and lipoamide have been identified as endogenous or dietary-derived substrates of FMOs in vitro. Using experimental models of knockout-mouse, they reveal previously unsuspected roles for FMOs in endogenous metabolic processes. FMO1 is identified as a novel regulator of energy balance that acts to promote metabolic efficiency; FMO5 is identified as a regulator of metabolic ageing and glucose homeostasis. This review demonstrates that FMOs do not function solely as xenobiotic-metabolizing enzymes but that any exposure to drugs and environmental chemicals that are substrates or inducers of FMOs would perturb the endogenous functions of these enzymes.

In the second review, Catucci et al. [2] look at Trimethylamine N-Oxide (TMAO), the product of the monooxygenation reaction catalysed by human flavin-containing monooxygenase 3 (hFMO3), and its animal orthologues. TMAO has recently been found to be related to several human health conditions such as cardiovascular and renal diseases. The authors discuss how TMAO enzymatic production and FMO catalytic activity are interconnected, answering to the interesting question of "enzymatically produced trimethylamine N-oxide: conserving it or eliminating it".

Third review of this special issue (Thodberg and Neilson [3]) deals with a very new topic: plant FMOs. To date, the majority of functional characterisation studies of FMOs have been performed on mammalian, fungal and bacterial FMOs with very limited research on plant FMOs. This is a timely review addressing the gap in our knowledge regarding plant FMOs; plants possess a far greater number of FMOs compared to bacteria and other eukaryotes. The authors focus on FMO diversity and functionality in the oxygenation reactions that are crucial steps within hormone metabolism, pathogen resistance, signalling and chemical defence. They demonstrate the fundamental role played by FMOs in plant metabolism, and more importantly their possible downstream applications such as their potential for the generation of novel bioactive metabolites. The review highlights the unique capabilities of plant FMOs that can be exploited for generation of valuable biocatalysts that perform unique reactions, relevant for the biotechnological and pharmaceutical industries.



**Citation:** Gilardi, G.; Sadeghi, S.J. Editorial: Special Issue on "Flavin Monooxygenases". *Catalysts* **2021**, *11*, 69. https://doi.org/10.3390/catal11010069

Received: 29 December 2020
Accepted: 3 January 2021
Published: 6 January 2021

Regarding the research articles of this special issue, they are all focused on the Baeyer-Villiger reactions which are another hallmark of these monooxygenase enzymes where an oxygen atom is inserted next to a carbonyl moiety. In the paper by de Souza et al. [4], the authors discuss the multi-drug resistance of Mycobacterium tuberculosis, the causative agent of tuberculosis. The treatment of this infection requires a complex regimen of antibiotics which are prodrugs that require activation by Baeyer-Villiger monooxygenases (BVMOs). BVMO EthA is known to activate the antitubercular drug ethionamide but some mutations of this BVMO enzyme have been isolated in patients with multi-drug resistance. The authors perform in silico docking experiments of ethionamide using a 3D model of EthA enzyme that they generated. Subsequently, they perform free energy computational calculations and demonstrate that the mutant EthA have reduced complex stability. Their results shed light on the molecular basis of ethionamide resistance.

In the second research paper by Niero et al. [5], the unique features of a new BVMO from an extreme halophilic archaeon, *Haloterrigena turkmenica* (*Ht*BVMO), is discussed. In recent years, numerous novel BVMOs have been identified leading to a continuous expansion of the enzymatic toolbox for their potential industrial application. Nevertheless, the present set of BVMOs is still far from meeting all industrial demands since they do not withstand industrial reaction conditions involving high temperatures, an acidic or alkaline environment or the presence of organic solvents. The latter prompted these authors to search for robust BVMOs in microorganisms able to thrive in extreme environments. To this end, they purified and characterised *Ht*BVMO which is the first BVMO showing typical features of halophilic proteins: salt-dependent activity and denaturation at low salt concentrations. In addition, this BVMO shows a very high number of negatively charged amino acids in its primary sequence, resulting in a net charge of −73 that is unique among known BVMOs and suggests this enzyme to be both halophilic and alkalophilic. This enzyme was also demonstrated to be active in the presence of solvents such as methanol and dioxane. The authors conclude that their characterisation of *Ht*BVMO can lead to engineering biocatalysts capable of functioning under harsh conditions similar to those routinely used in industry.

Staying with the industrial application theme of BVMOs, Tolmie et al. [6] discuss the natural variation in the "control loop" of another BVMO, $BVMO_{AF210}$ from *Aspergillus flavons* and its influence on regioselectivity and sulfoxidation. The authors note that with the exception of directed evolution studies influencing the substrate scope and selectivity/specificity through allosteric effects, protein engineering of BVMOs to improve substrate acceptance or regio-, enantio-, and stereospecificity/selectivity has mostly focused on "hot spot" residues lining the active site. However, in their study they point to another region within the protein which might have a similar role in stereospecificity/selectivity, that of the control loop between the two domains of the protein. This 'control loop' was shown to not only have a direct impact on the overall structure but also the active site environment of several BVMO proteins. The authors mutate a specific residue Thr513 within this loop to several different amino acids. Although the mutagenesis of this amino acid residue did not significantly alter the substrate scope, changes in the reaction rates were observed. The mutation of this position also brought about changes in the regio- and enantioselectivity of the enzyme, for example mutations to the bulkier amino acids either significantly improved the regioselectivity for the normal lactone production from 2-methylcyclohexanone or allowed access to more of the abnormal lactone from 2-methylcyclopentanone. Moreover, lower rates of overoxidation during sulfoxidation of thioanisole were also observed.

In the final research article, the group of Prof. Mihovilovic [7] tackle biocatalysis with the combination of enzymes in cascade reactions. Several whole-cell enzyme cascade systems using the effect of shifting the reaction equilibrium using an irreversible reaction catalysed by BVMOs, have been previously reported to maximise the yield of the desired product. The authors went one step further and developed an immobilization process for recombinant *E. coli* cells harbouring the multi-enzymatic cascade system of alcohol dehydrogenase, enoate reductase and cyclohexanone monooxygenase. The operational

stability of the free and immobilised cells with the enzyme cascade was assessed with significant improvements of operational stability and product yield demonstrated by the immobilization of cells in PEC beads.

In conclusion, the present Special Issue covers recent progress not only in the possible new applications of these enzymes as biocatalysts, but also their emerging roles in human health and disease. It is clear from the different contributions that the reason behind the popularity of these monooxygenase enzymes is (a) the vast array of reactions that they can catalyse, including Baeyer-Villiger oxidation, sulfoxidation, epoxidation and N-oxidations as well as (b) their high selectivity in chemo-, regio-, and enantio-selective oxygenation reactions. Further research on plant FMOs will most probably change the currently somewhat simplistic view of FMOs as broad substrate-accepting detoxifying enzymes, as the plant enzymes appear to have highly specific physiological roles in the biosynthesis of various small molecules. Finally, the future holds many promises for these flavin-containing monooxygenases in wide applications in various fields including high-value fine chemicals, cosmetics as well as drug metabolites in the pharmaceutical industries.

**Funding:** This research received no external funding.

**Informed Consent Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Phillips, I.R.; Shephard, E.A. Endogenous Roles of Mammalian Flavin-Containing Monooxygenases. *Catalysts* **2019**, *9*, 1001. [CrossRef]
2. Catucci, G.; Querio, G.; Sadeghi, S.J.; Gilardi, G.; Levi, R. Enzymatically Produced Trimethylamine N-Oxide: Conserving It or Eliminating It. *Catalysts* **2019**, *9*, 1028. [CrossRef]
3. Thodberg, S.; Neilson, E.H.J. The "Green" FMOs: Diversity, Functionality and Application of Plant Flavoproteins. *Catalysts* **2020**, *10*, 329. [CrossRef]
4. De Souza, V.C.; Antunes, D.; Santos, L.H.S.; Goliatt, P.V.Z.C.; Caffarena, E.R.; Guimarães, A.C.R.; Galvão, T.C. Insights into the Mechanism of Ethionamide Resistance in Mycobacterium tuberculosis through an in silico Structural Evaluation of EthA and Mutants Identified in Clinical Isolates. *Catalysts* **2020**, *10*, 543. [CrossRef]
5. Niero, M.; Righetto, I.; Beneventi, E.; de Laureto, P.P.; Fraaije, M.W.; Filippini, F.; Bergantino, E. Unique Features of a New Baeyer—Villiger Monooxygenase from a Halophilic Archaeon. *Catalysts* **2020**, *10*, 128. [CrossRef]
6. Tolmie, C.; Aido-Machado, R.d.; Ferroni, F.M.; Smit, M.S.; Opperman, D.J. Natural Variation in the 'Control Loop' of BVMOAFL210 and Its Influence on Regioselectivity and Sulfoxidation. *Catalysts* **2020**, *10*, 339. [CrossRef]
7. Bučko, M.; Gemeiner, P.; Krajčovič, T.; Hakarová, M.; Chorvát, D.; Chorvátová, A.M.; Lacík, I.; Rudroff, F.; Mihovilovič, M.D. Immobilized Cell Physiology Imaging and Stabilization of Enzyme Cascade Reaction Using Recombinant Cells Escherichia coli Entrapped in Polyelectrolyte Complex Beads by Jet Break-Up Encapsulator. *Catalysts* **2020**, *10*, 1288. [CrossRef]