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Description of the funded research project

1st Call for H.F.R.I. Research Projects to Support Faculty Members & Researchers and Procure High-Value Research Equipment

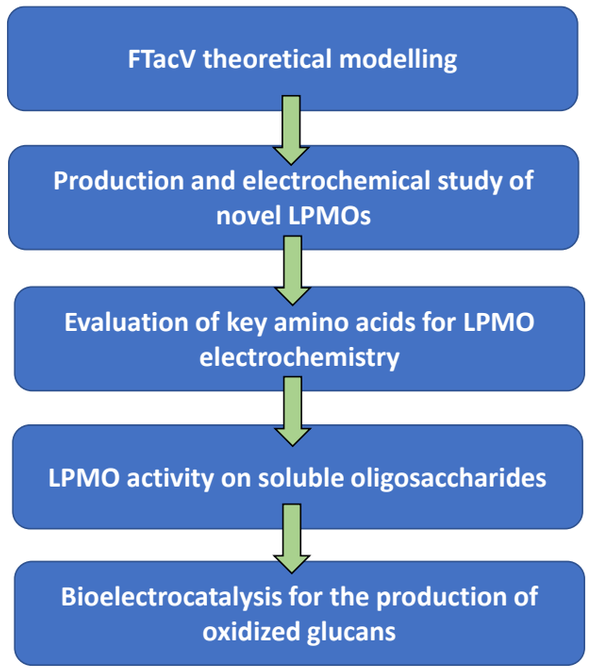
Title of the research project: Electrochemical Analysis for the action of Lytic Polysaccharide Monooxygenases

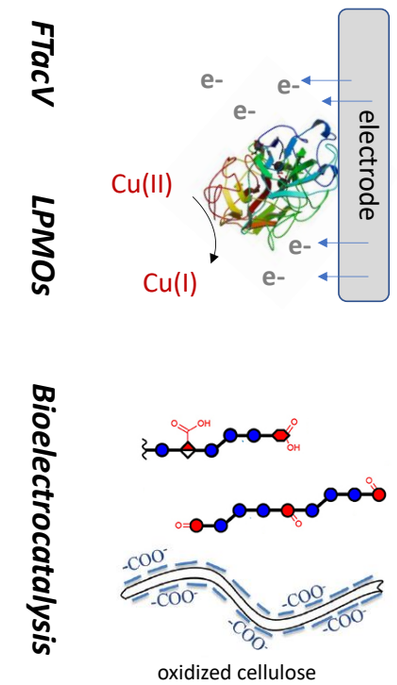
Principal Investigator: Antonios Karantonis

Reader-friendly title: Echem-4-LPMO

Scientific Area: Engineering Sciences & Technology

Host Institution: School of Chemical Engineering, National Technical University of Athens, Greece





Budget: 190.000 €

Duration: 36 months

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Research Project Synopsis

Echem-4-LPMO

The aim of the research project **Echem-4-LPMO** is to employ the most recent relevant methods in the field of electrochemistry to study the mechanism of action of the newly discovered *lytic polysaccharide monooxygenases (LPMO).*

By introducing a novel, large amplitude alternate current cyclic voltammetry (**FTacV**) combined with a simple and easy to perform enzyme immobilization, **Echem-4-LPMO** will attempt to clarify the mechanism of action of LPMO enzymes that contribute to the enhancement of hydrolytic cleavage of polysaccharides, and to elucidate the conditions under which this catalytic action is optimized. By this way, **Echem-4-LPMO** will move the field of LPMO study of mechanism of action well beyond state-of-the-art by introducing a novel method to track the direct electron transfer and shed light on different aspects of this enzyme's class mechanism.

The project includes the following steps: (**1**) software development for the signal processing of FTacV, (**2**) modelling of FTacV parameters, (**3**) development of kinetic models of LPMOs using FTacV, (**4**) expression and production of novel fungal thermoactive LPMOs, (**5**) immobilization of LPMOs onto the electrode surface, (**6**) experimental determination of (apparent) standard potential and redox kinetic constants, (**7**) determination of parameters affecting the enzymes performance, (**8**) study of the catalytic activity of the enzyme on oligo- and poly-saccharides as substrates in the presence of O_2 or H_2O_2, (**9**) study of the effect of mutations on LPMO action and (**10**) bioelectrocatalysis using immobilized LPMOs onto electrode probes

Project originality

Echem-4-LPMO

The novel aspects of this work consist in the approach to study the electrochemical behavior of redox enzymes. Although kinetic studies have been done in the past using redox enzymes, no attempts have been made for the study thought ***direct electron transfer by monitoring directly the interaction of the active site of enzymes with an electrode surface and the corresponding substrates***. Moreover, no systematic approach has been made to correlate FTacV with its parameters, nonetheless correlate them with the extraction of kinetic constants of a catalytic reaction.

The ***novel aspects*** of the **Echem-4-LPMO** project are: (**1**) the electrochemical investigation of the action of LPMOs, (**2**) the implementation of a new technique for the experimental study of LPMOs, (**3**) the combination of new simulation results with new experimental findings, (**4**) the development of protocols for the implementation of a new experimental technique in bioelectrochemistry, (**5**) the development of a dedicated signal processing software and (**6**) the investigation of bioelectrocatalytic action of LPMOs towards the production of oxidized products (oligosaccharides and glucans) with great properties and numerous applications, in the absence of any external reducing agent.

Expected results & Research Project Impact

Echem-4-LPMO

The expected results of the project include: (**1**) the development of a tailor-made FTacV methodology for the study of the electrochemical behavior of LPMOs, by achieving a reliable and complete modelling with FTacV that gives reproducible results and similar to the experimental outcome, (**2**) expression and characterization of at least two novel active fungal LPMOs, (**3**) elucidation of role of O_2 and H_2O_2 as co-substrates on the electrochemistry of LPMOs, (**4**) isolation and FTacV study of at least two LPMO mutants with altered electrochemical behavior, (**5**) efficient immobilization of LPMOs on the electrode that retain their catalytic activity and finally (**6**) successful bioelectrocatalysis of soluble oligosaccharides and insoluble glucans and nanocellulose by LPMOs.

Regarding the *impact* of the **Echem-4-LPMO** project, it can be divided into two different aspects. The first one is the fact that *bioelectrocatalysis* which is the ultimate scope of this project is an eco-friendly process since the addition of external chemicals is omitted as the electron supply is the electrode surfaces which are reusable. Since LPMOs comprise an integral component of commercial enzyme preparations for efficient biomass degradation and they have been shown to increase the performance of carbohydrate hydrolases up to 10-times, the understanding of their mechanism can pave the way for their use in bioelectrocatalysis processes, to limit the use of harsh chemicals. The other aspect refers to the fact that the successful implementation **Echem-4-LPMO** project can lead to answers regarding some very important issues that concern the industry and will also be a declaration of the importance of electrochemistry in the study of redox enzymes.

The importance of this funding

Echem-4-LPMO

The funding of this project by H.F.R.I will enable improving innovation capacity, as well as the continuation of our work on bioelectrocatalysis and integration of new knowledge. **Echem-4-LPMO** aims to address fundamental questions on a new conceptual basis, while integrating the previous results of our group, in order to shed light on different mechanistic aspects regarding the mode of action of LPMOs.

The successful implementation of the project requires the complementarity and the knowhow of different fields (electrochemistry/engineering combined with biochemistry/biocatalysis). This funding will give the opportunity to young researchers with high scientific potential working at NTUA not only to undertake research with technological support and combine their expertise towards the accomplishment of the research and technology targets of the **Echem-4-LPMO** project, but also to participate in conferences, expand the network of international collaborations and be otherwise productive.

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