Structural and mechanistic characterization of lytic polysaccharide monooxygenases (LPMO-s) from thermophilic Actinobacteria

Summary

Lytic polysaccharide monooxygenases (LPMO-s) are an important class of biotechnological enzymes that play a crucial role in the second generation biorefineries. It's been estimated that the cellulose-degrading enzyme cocktails used by today's biotech industry are made up of close to 20% of LPMO-s by weight. Since many technological processes occur at elevated temperatures and extreme pH environments, there has been a rise in interest to sourcing those enzymes from organisms that can withstand extreme conditions (high temperature, alkalinity, etc.). The primary goal of current doctoral thesis is to isolate and characterization of LPMO-s from thermophilic Actinobacteria utilizing structural biology and enzymology methods. The overall goal of the project is to provide a thorough insight into the structural and functional elements that are involved in ensuring both temperature stability as well as substrate specificity of the enzymes under study. Although in silico methods have come a long way (e.g. AlphaFold) accurate validation of structural nuances within a proposed model can only be achieved by the utilization of physical structural biology methods (X-ray crystallography or CryoEM). The kinetic models for these enzymes can be rather complex due to the requirement of hydrogen peroxide and an external source of electrons (e.g. through the coupling to oxidation of lignin derived phenolics) to carry out effective cleavage of glycosidic bonds within the crystalline cellulose polymer matrix. Therefore, tweezing out the details with respect to the kinetic models of these enzymes for their utilization in complex enzyme mixtures that are commonly used by industry, will be paramount.

Research field:	Chemistry and biotechnology
Supervisor:	Dr. Tiit Lukk
Availability:	This position is available.
Offered by:	School of Science Department of Chemistry and Biotechnology
Application deadline:	Applications are accepted between June 01, 2022 00:00 and June 30, 2022 23:59 (Europe/Zurich)

Description

The doctoral candidate will be involved in all aspects of the extremophile LPMO-related work: designing codon-optimized DNA constructs; expressing and purifying the proteins from a heterologous expression system; deciphering the kinetic mechanisms of these enzymes as well as solving high-resolution X-ray structures of the LPMO-s. Since many cellulose-acting enzymes are modular (incl. LPMO-s), the doctoral student will be responsible for characterization of not only the catalytic portion of the enzyme, but also the carbohydrate binding modules (CBM-s), which are tethered to the catalytic domain via a long flexible linker. Since many cellulose-acting enzymes are inhibited by lignin or lignin-derived phenolics, the project will attempt to answer the question of weather the LPMO-s derived from extremophiles are inactivated or conversely activated by the presence of biomass-derived phenolics.

The thesis should address the following questions:

1. Can the LPMO-s from extremophilic organisms in fact better withstand elevated temperatures than the commercially (fungal) available LPMO-s?
2. What are the effective pH activity ranges of the LPMO-s from the thermoalkaliphilic *Cellulomonas sp.* FA1 organism?
3. What are the key structural elements responsible for defining substrate specificity?
4. What effect does the source of biomass and the composition of phenolics have on the activity of the LMPO-s under study?

Responsibilities and (foreseen) tasks

- Carry out molecular biology and microbiology work related to the project
- Express, purify and crystallyze enzymes under study
- Solve the X-ray structures of LPMO catalytic domains and CBM-s, carry out modeling of full-length bimodular LPMO-CBM enzymes by the utilization of Small Angle X-ray Scattering
- Carry out thorough kinetic analysis and build kinetic models of the enzymes
- Publish all of the aforementioned work in a Q1 journal

Applicants should fulfil the following requirements:

- a master's degree in biological sciences (preferably in biochemistry or bioorganic chemistry)
- a clear interest in the topic of the position
- excellent command of English
- strong and demonstrable writing and analytical skills
- capacity to work both as an independent researcher and as part of an international team
- capacity and willingness to provide assistance in organizational tasks relevant to the project

The following experience is beneficial:

- Microbiology and protein work (expression, purification)
- Experimental and/or theoretical biophysics knowledge
- Protein crystallization experience
- Experience in enzyme kinetics

The candidate should submit a research plan for the topic, including the overall research and data collection strategy. The candidate can expand on the listed research questions and tasks, and propose theoretical lenses to be used.

About the department

The department of Chemistry and Biotechnology was created in the School of Science in 2017, founding director was Ivar Järving. The institute merged the former Institute of Chemistry and the Institute of Gene Technology from the Faculty of Mathematics and Natural Sciences and the Institute of Food Science from the Faculty of Chemistry and Materials Technology. Recent scientific discoveries in chemistry, molecular biology and food technology have opened up completely new perspectives in fields as medicine, industry, agriculture and the environment.

Additional information

For further information, please contact Dr Tiit Lukk tiit.lukk@taltech.ee or visit https://puidukeemia.ee/tooruhmad/bio-massi-biokeemiline-vaarindamine/.

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