Functional characterization of intrinsically disordered regions in fungal LPMOs

Keywords
Lytic polysaccharide monooxygenases (LPMOs), fungi, lignocellulose degradation, conformational disorder, enzymes, fuzzy appendages

Summary
Lytic polysaccharide monooxygenases (LPMOs) are copper-containing enzymes encountered in bacteria and filamentous fungi that have revolutionized the enzymatic degradation of recalcitrant polysaccharides (chitin, cellulose, starch) (Tandrup et al., Biochem Soc Trans 2018). These enzymes have a great biotechnological interest and are important component of industrial enzyme cocktails used for the production of bioproducts (e.g. bioethanol) from lignocellulosic biomass. A bioinformatic analysis carried out on fungal LPMOs from different CAZy families revealed that their C-terminal extension is predicted to be intrinsically disordered, but the functional relevance of these peculiar features is unknown. Intrinsically disordered regions (IDRs) are devoid of stable secondary and tertiary structure and therefore exist as highly dynamic ensembles (Habchi et al., Chem Rev 2014). Since IDRs are typically involved in regulation and signaling cellular processes, it is tempting to hypothesize that this C-terminal extension may play a regulatory role in LPMOs from filamentous fungi.

The objectives of this project consist in assessing (i) the actual structural state of the disordered Cterminal domain (dCTD) predicted to be intrinsically disordered, (ii) possible mutual conformational effects between the structured catalytic domain and the dCTD and (iii) the functional impact of the dCTD on LPMO function. To reach these objectives, two teams (BBF and AFMB) with a distinct and complementary expertise will combine their efforts in the context of a new collaborative project.

The supervisor has a sound expertise in the characterization of fungal LPMOs in the context of plant cell wall degradation (Couturier et al., Nat Chem Biol 2018; Filiatrault-Chastel et al., Biotechnol Biofuels 2019; Labourel et al., Nat Chem Biol 2020). The co-supervisor has a well-recognized expertise in the identification, purification and characterization of IDRs and of the interactions they establish with their partners (http://www.afmb.univ-mrs.fr/structural-disorder-and-molecular). A panel of biochemical and biophysical approaches will be used to characterize LPMOs dCTD. The impact of post-translational modifications, and in particular O-glycosylation and phosphorylation, on the conformational properties of the dCTD will also be investigated. These complementary approaches will provide insights into the function of LPMO dCTD with fundamental and biotechnological outcomes.

The co-supervisors

Jean-Guy BERRIN, Biodiversité et Biotechnology Fongiques (BBF) – jean-guy.berrin@inrae.fr
Sonia LONGHI, Architecture et Fonction des Macromolécules Biologiques (AFMB)– sonia.longhi@afmb.univ-mrs.fr

Location
BBF and AFMB Laboratories, Luminy Campus, Marseille, France

Doctoral school
Life and Health Sciences (ED 62), Aix-Marseille université

Expected profile of the candidate
Theoretical and practical knowledge in biochemistry (expression in heterologous systems and purification of recombinant proteins). Theoretical (and ideally also practical) knowledge on intrinsically disordered proteins and/or in enzymology. Theoretical (and ideally also practical) knowledge in far-UV circular dichroism, analytical size exclusion chromatography, and fluorescence spectroscopy.