

Title of the PhD project:

GlycoWall: Molecular insight into yeast cell wall polysaccharide synthesis

PhD supervisors:

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<https://www.ibs.fr/research/research-groups/structure-and-activity-of-glycosaminoglycans-group/atip-junior-team-r-wild/>

Project summary:

The cell wall is a protective and rigid layer and is thus an important factor in maintaining cellular integrity. The major components of the yeast cell wall are the polysaccharides chitin, glucan and mannan. Chitin is synthesized by a plasma membrane-localized glycosyltransferase, which also translocates the growing polysaccharide chain into the extracellular matrix. The proposed PhD project focuses on the functional and structural characterization of the fungal chitin synthase CHS1, which catalyzes the step-wise addition of N-acetylglucosamine moieties to a growing polysaccharide chain. To gain insight into the architecture of this large, membrane-integral protein and the catalyzed glycan transfer reaction our aim is to determine a high-resolution structure of CHS1 using single-particle cryo-electron microscopy and to develop an in vitro activity assay to follow polysaccharide chain elongation and translocation across the plasma membrane. Preliminary experiments show that the CHS1 protein can be expressed using a mammalian expression system and the PhD student will optimize the purification procedure of the membrane protein, including screening of different detergents for solubilization. An in vitro activity assay will be developed to check activity of purified proteins. Reconstitution of CHS1 into liposomes will allow to study translocation of the produced polysaccharide chain across the membrane. The student will learn how to prepare samples for cryo-electron microscopy experiments and how to collect and analyze the data. The obtained structural model will be validated by characterizing structure-based mutants in vitro and in vivo.

Preferred skills:

- Master in molecular biology, biochemistry or structural biology
- Demonstrated experience in expressing, purifying and characterizing proteins
- First experience in high-resolution structure determination would be an advantage
- Good written and oral communication skills in English

Student role:

- Cloning, expression and purification of membrane proteins
- Establish in vitro glycosyltransferase assays
- High-resolution structure determination using cryo-EM, data analysis and model building
- Functional analysis using of yeast knock-out strains
- Writing peer-reviewed manuscripts and presenting results at international conferences

Keywords:

Structural biology, Cryo-electron microscopy, Membrane protein, Yeast, Glycobiology

Relevant publications of the team:

1. Neuhaus JD*, Wild R**#, Eyring J, Irobalieva RN, Kowal J, Lin C-W, Locher KP, Aebi M* (2021). Functional analysis of Ost3p and Ost6p containing yeast oligosaccharyltransferases. **Glycobiology** doi: 10.1093/glycob/cwab084.
2. Annaval T., Wild R., Crétinon Y., Sadir R., Vivès R.R. and Lortat-Jacob H (2020). Heparan sulfate proteoglycans biosynthesis and post synthesis mechanisms combine few enzymes and few core proteins to generate extensive structural and functional diversity. **Molecules** doi: 10.3390/molecules25184215.
3. Debarnot C., Monneau Y.R., Roig-Zamboni V., Delauzun V., Le Narvor C., Richard E., Hénault J., Goulet A., Fadel F., Vivès R.R., Priem B., Bonnaffé D., Lortat-Jacob H., Bourne Y (2019). Structural insights into substrate binding and catalytic mechanism of human heparan sulfate D-glucuronyl C5 epimerase. **PNAS** doi: 10.1073/pnas.1818333116.
4. Wild R*, Kowal J*, Eyring J*, Ngwa EM, Aebi M, Locher KP (2018). Structure of the yeast oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation. **Science** doi: 10.1126/science.aar5140.
5. Wild R*, Gerasimaite R*, Jung JY*, Truffault V, Pavlovic I, Schmidt A, Saiardi A, Jessen HJ, Proirier Y, Hothorn M, Mayer A (2016). Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. **Science** doi: 10.1126/science.aad9858.