Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
Rebekka Wild, rebekka.wild@ibs.fr

Host laboratory:
Institut de Biologie Structurale
https://www.ibs.fr/spip.php?lang=en

Title of the M2 research internship:
Structural and functional characterization of the glycosaminoglycan biosynthesis machinery

Project summary:
The proposed M2 project focuses on the molecular characterization of the EXT1-EXT2 complex, a key enzyme complex in glycosaminoglycan biosynthesis. Glycosaminoglycans are long linear polysaccharides that are found on the cell surface and in the extracellular matrix of all animal cells. They are covalently attached to serine residues of core proteins, thereby regulating the interaction with growth factors, signaling receptors, cytokines, adhesion molecules and diverse matrix components. Malfunctioning of glycosaminoglycan biosynthesis has been linked to Alzheimer's disease, acute and chronic inflammation, tumorigenesis and diabetes.

The EXT1-EXT2 complex carries out the glycosaminoglycan chain elongation reaction by catalyzing the alternating addition of N-acetylglucosamine and glucuronic acid molecules. To gain insight into the architecture of the EXT1-EXT2 complex, the molecular mechanism of substrate recognition and the catalyzed glycan transfer reactions, the aim of the project will be to determine a high-resolution structure using single-particle cryo-electron microscopy.

Preliminary protein over-expression experiments indicate that the two proteins can be co-expressed in HEK293 cells. The M2 student will optimize the purification procedure of this Golgi-localized, membrane-anchored complex. This will include screening of different detergents for solubilization and determining optimal buffer conditions by analyzing sample stability using nanoscale differential scanning fluorimetry (nanoDSF). In vitro glycosyltransferase assays will be carried out to ensure that the purified complex is catalytically active. Finally, sample quality will we assessed using negative-stain and cryo-electron microscopy. Once suitable conditions are found, a data set at a high-end cryo-electron microscope will be collected to determine a structure of the EXT1-EXT2 complex de novo without the prior need of structural information from X-ray crystallography. The student will be closely supervised to learn how to prepare samples for cryo-electron microscopy experiments, how to collect and analyze this data and build a structural model.

Keywords:
glycosaminoglycan biosynthesis, enzyme complex, single-particle cryo-electron microscopy

Relevant publications of the team:
Annaval T, Wild R, Crétinon Y, Sadir R, Vivès RR, 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 25(18):4215.

Wild, R., Kowal, J., Eyring, J., Ngwa, E.M., Aebi, M., and Locher, K.P. (2018). Structure of the yeast oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation. Science 359, 545–550.

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). Substrate binding mode and catalytic mechanism of human heparan sulfate d-glucuronyl C5 epimerase. PNAS 116, 6760–6765.

Pegeot, M., Sadir, R., Eriksson, I., Kjellen, L., Simorre, J.-P., Gans, P., and Lortat-Jacob, H. (2015). Profiling sulfation/epimerization pattern of full-length heparan sulfate by NMR following cell culture 13C-glucose metabolic labeling. Glycobiology 25, 151–156.