

**Master 2 internship project
Year 2020-2021**

Laboratory/Institute: IBS
Team: SAGAG

Director: Winfried Weissenhorn
Head of the team: Hugues Lortat-Jacob

Name and status of the scientist in charge of the project: Rebekka Wild
yes **no**

HDR:

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Program of the Master's degree in Biology:

- Immunology, Microbiology, Infectious Diseases Integrative Structural Biology
 Physiology, Epigenetics, Differentiation, Cancer Neurosciences and Neurobiology
 Planta International

Title of the project: Molecular insight into heparan sulfate chain elongation

Objectives (up to 3 lines):

This M2 proposal aims for the functional and structural characterization of the EXT1-EXT2 enzyme complex. This membrane-anchored complex catalyzes the consecutive addition of sugar molecules to a core protein and therefore plays a key role in heparan sulfate biosynthesis.

Abstract (up to 10 lines):

Heparan sulfates are long linear polysaccharides that are found on the cell surface and in the extracellular matrix. They are covalently attached to serine residues of core proteins, thereby regulating their interaction with growth factors, signaling receptors, cytokines, and many others. To gain insight into the architecture of the EXT1-EXT2 complex and the catalyzed polysaccharide chain elongation reaction, we wish to determine a high-resolution cryo-electron microscopy structure of this complex. 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 the membrane complex, which will include screening of different detergent and buffer conditions. Next, *in vitro* glycosyltransferase assays will be carried out to ensure that the purified complex is catalytically active. In close collaboration with a senior researcher, the student will prepare samples for cryo-electron microscopy experiments and learn how to collect and analyze this data.

Methods (up to 3 lines):

Purification of membrane protein complex using different chromatography techniques
Protein characterization (Western Blot, SDS Page, protein stability by nanoDSF and activity assays)
Cryo-electron microscopy experiments (sample preparation, data collection and processing)

Up to 3 relevant publications of the team:

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.

Wild, R., Kowal, J., Eyring, J., Ngwa, E.M., Aebi, M., and Locher, K.P. (2018). Structure of the yeast

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oligosaccharyltransferase complex gives insight into eukaryotic N-glycosylation. Science 359, 545–550.

Requested domains of expertise (up to 5 keywords):

Experience or interest in molecular biology, biochemistry and structural biology. Good English skills.