

**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 <sup>13</sup>C-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

## **Master's degree in Biology – Chemistry-Biology Department**

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.