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

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Title of the M2 research internship:
Cross-linking of Heparan Sulfate induced by CXCL12 chemokine : structural remodelling of glycocalyx and functional effects

Project summary:
The glycocalyx or “sugar coat” consists of complex polysaccharides and proteins and its impairment is associated with many diseases related to chronic inflammation. Heparan sulfate (HS) is the major glycosaminoglycan component of the endothelial surface glycocalyx. This complex polysaccharide binds many signalling molecules such as chemokines and growth factors, thereby modulating their structure, stability, localization and functions. Previously, using a combination of biochemical/biophysical techniques (QCM-D and FRAP) in a well-defined biomimetic surfaces, we showed that HS binding chemokine (CXCL12) and growth factor (FGF-2) cross-link HS chains, reduce the thickness of the hydrated layers they form, and change their mobility and dynamics. These data describe a new paradigm of protein/HS interactions by revealing that HS-binding proteins could regulate the structure and the organization of HS chains.
In this project, we propose to examine, in cell context, the architecture, the spatial organization of HS upon chemokine binding and to investigate the functional effects of this structural remodelling.
For that purpose, we will study (I) the ability of CXCL12 to induce the HS nanoclusters by using super resolution microscopy (STORM / 2D and 3D imaging) and (II) the dynamic changes of adhesive sites. Our working hypothesis is that CXCL12-induced HS nanoclusters through cross-linking will affect adhesion molecules accessibility (e.g ICAM-1) and plasma membrane topography. Therefore, we will study cell mechanics (P-myosin and actin staining) and cell adhesive/migratory behaviour (distribution and dynamics of adhesive sites using FRAP (GFP-vinculin and GFP-integrin) / cell migration assay). Preliminary results, using confocal microscopy, allowed us to observe that CXCL12 changes HS spatial distribution at the endothelial cell surface, providing a solid basis for the project.
The Master 2 student will learn and prepare samples for single molecule super-resolution imaging (cell culture, immunolabelling assay,...), and will perform different functional assays (Flow cytometry, Cell Adhesion and Cell Migration/chemotaxis assay).

Keywords:
heparan sulfate/proteins interactions, super-resolution microscopy, inflammatory disease

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. Structural insights into substrate binding and catalytic mechanism of human heparan sulfate D-glucuronyl C5 epimerase. Proc. Natl. Acad. Sci. USA 116, 6760-6765 (2019)

Connell B.J., Sadir R., Baleux F., Laguri C., Kleman J-P., Luo L., Arenzana-Seisdedos F. and Lortat-Jacob H. Heparan Sulfate differently regulates CXCL12α and CXCL12γ mediated chemotaxis through differential presentation to CXCR4. Science Signaling 9, ra107 (2016)

Liu X Q, Fourel L, Dalonneau F, Sadir R, Leal S, Lortat-Jacob H, Weidenhaupt M, Albiges-Rizo C, Picart C. Biomaterial-enabled delivery of SDF-1α at the ventral side of breast cancer cells reveals a crosstalk between cell receptors to promote the invasive phenotype. Biomaterials, 127:61-74 (2017).

Migliorini E, Thakar D, Kühnle J, Sadir R, Dyer D, Volkman B, Handel T, Lortat-Jacob H, Fernig D, Coche-Guerente L, and Richter RP. Cytokines and Growth Factors cross-link heparan sulfate. Open Biol. 10.1098/rsob.150046 (2015)