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International PhD Program IC-PhD

PhD thesis project

2024 Call for application

The molecular machinery of galectin-driven endocytosis

General information

Call	2024
Reference	2024-16-WUNDER_JOHANNES
Keyword(s)	Cell biology; DNA-origami; glycobiology; GL-Lect driven endocytosis; mass spectrometry

Director(s) and team

Thesis director(s)	Ludger Johannes & Christian Wunder
Research team	Endocytic Trafficking and Intracellular Delivery
Research department	UMR3666 / U1143 - Cellular and Chemical Biology

Description of the PhD thesis project

Abstract

The internalization of extracellular and plasma membrane-localized molecules is critical for various physiological functions such as signaling, cell migration, membrane turnover, neurotransmission, metabolism, and pathogen invasion.

Several uptake mechanisms exist and act in parallel. Clathrin-mediated endocytosis is the best-characterized internalization pathway with a well-defined sequence of events and molecular machinery. Clathrin-independent endocytosis processes have also been documented, but these remain poorly explored (Building endocytic pits without clathrin, Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis). GlycosLipid-Lectin (GL-Lect)-driven endocytosis was pioneered in our team and belongs to the group of clathrin-independent endocytic processes.

It remains unexplored to what extent different GL-Lect driven endocytic processes share the same molecular identity (glycoproteins, glycolipids and glycans). We hypothesize the existence of a glycocode that determines the final fate of internalized cargo molecules.

With this PhD-project, we aim to test the glycocode hypothesis using our newly developed DNA-based nanotechnology for spatio-temporal multiomics.

The French or non-French PhD student will acquire and develop a broad range of expertise in molecular biology, recombinant protein purification, cell biology, analysis of omics results, lattice light-sheet microscopy and electron microscopy. The PhD student will benefit from mentoring and/or a secondment abroad through a collaborative network to gain additional training in glycobiology.

Background

Virtually all plasma membrane proteins are glycosylated. The activity of these glycoproteins is tightly regulated and depends on dynamic changes in the glycan pattern and subsequent endocytosis. Secreted extracellular lectins (galectins) recognize these glycans. Galectins belong to a family of 15 members (Galectins at a glance). We have pioneered the GlycoLipid-Lectin (GL-Lect) mechanism (Glycolipids and Lectins in Endocytic Uptake Processes) that triggers endocytosis (Galectin-3 drives glycosphingolipid-dependent

biogenesis of clathrin-independent carriers, Endophilin-A3 and Galectin-8 control the clathrin-independent endocytosis of CD166) and subsequent retrograde trafficking from endosomes to the Golgi in cells in culture (Persistent cell migration and adhesion rely on retrograde transport of β(1) integrin) and in mice (Glycolipid-dependent and lectin-driven transcytosis in mouse enterocytes). A cascade of events characterizes the GL-Lect mechanism: 1. Galectin binding to glycosylated cargo, 2. galectin oligomerization, 3. galectin-driven recruitment of glycosphingolipids, 4. glycosphingolipid-dependent membrane bending, and 5. endocytosis.
DNA-origami is an emerging field and has been used to build a variety of nucleotide-based nanostructures. We have previously applied DNA nanostructures to visualize endocytic uptake processes (Quantum dot-loaded monofunctionalized DNA icosahedra for single-particle tracking of endocytic pathways). With this PhD project, we aim to exploit a modified DNA-origami design to test by spatio-temporal multiomics the hypothesis on the existence of a glycocode underlying galectin-driven endocytic events.
Glycans play an important role in physiological and pathological conditions. Bacteria and viruses use glycans to invade host cells. Cancer cells have modified glycan landscapes that are currently being used to monitor cancer progression. Tumor-specific glycan patterns may be exploited to target cancer cells for biomedical applications.

Objectives
Spatio-temporal multiomics is a key approach to identify molecular players and understand complex biological processes. Our newly developed DNA nanotechnology-based method will allow us to deeply characterize endocytic processes in human cells.

We have three main objectives:
1) Purification of several members of the galectin family. Characterization of their endocytosis by means of light and electron microscopy.
2) Application of DNA-nanotechnology to key galectins for proteomics, (glycosphingo)lipidomics and glycomics/glycoproteomics.
3) Validation of key players identified by the omics approach using siRNA knock-down, CRISPR-knock out, and functional tests.

We thereby expect to establish glycosylation as a key determinant of endocytic uptake and to provide stringent testing of the existence of a glycocode for the sorting of endocytic cargoes to district populations of endocytic carriers.

Experimental approaches
The main experimental approaches are: Cloning, recombinant expression and purification of galectins, DNA-origami for multiomics, and lattice light-sheet and electron microscopy to characterize the different galectins and players (cargoes, trafficking machinery) identified in the multiomics approach.
The PhD student will benefit from the team's experience in the purification of galectins. In addition, the planned experiments will involve the use of a DNA nanotechnology-based method already established in our laboratory. The Curie Institute hosts several technology platforms that will be used for the outlined project, including proteomics, lipidomics and imaging (https://institut-curie.org/dispatch/curiecoretech-core-facilities). The PhD student will be supported by an experienced senior scientist and an electron microscopy engineer. In addition, complex 4D-image analysis of lattice light-sheet microscopy data will be performed with a mathematics team that recently joined our unit (https://team.inria.fr/serpico/).

International, interdisciplinary & intersectoral aspects of the project

The Ludger JOHANNES laboratory has an international collaborative network, which will be used by the PhD student to gain additional training in glycobiology (Copenhagen Center for Glycomics) and perform glycomics experiments (Glycoproteomics at Science for Life Lab (SciLifeLab)).

The project will be carried out in close contact with Galecto Inc, a biotech company focused on the development of specific galectin inhibitors (https://galecto.com). The student will benefit from their technical support and expertise. The project will generate approaches that will very likely be of great interest to the community and potentially to industrial partners. On the basis of the proposed PhD work, we intend to patent strategies to interfere with specific endocytic processes. For this, we will be supported by the Technology Transfer Office of the Curie Institute (https://techtransfer.institut-curie.org/).
The PhD student will acquire and develop a broad range of expertise. The PhD program is interdisciplinary by nature, linking cell biology including advanced imaging and biocomputational image analysis, DNA-nanotechnology, multiomics, and glycobiology. The focus can be modulated according to personal preferences. The doctoral student is thus trained to become an independent scientific thinker at the interface between the various disciplines.
In addition, the Advanced Training Office of the Curie Institute (https://training.institut-curie.org/) is providing seminars and workshops to prepare PhD-students and post-docs for their next career steps (paper- and grant-writing, applying for academia and industry, patenting, alternative careers in research, team-building …).

Recent publications

1. Billet, A., J. Hadjerci, T. Tran, P. Kessler, J. Ulmer, G. Mourier, M. Ghazarian, A. Gonzalez, R. Thai, P. Urquia, A.C. Van Baelen, A. Meola, I. Fernandez, S. Deville-Foillard, E. MacDonald, L. Paolini, F. Schmidt, F. Rey, M.S. Kay, E. Tartour, D. Servent, and **L. Johannes**. 2023. A synthetic delivery vector for mucosal vaccination. ***Biomaterials***. 2023 Nov;302:122298.

2. Lucchino, M., A. Billet, S.K. Bai, E. Dransart, J. Hadjerci, F. Schmidt, **C. Wunder, and L. Johannes**. 2021. Absolute Quantification of Drug Vector Delivery to the Cytosol. ***Angew. Chem. Int. Ed. Engl.*** 60:14824-14830.

3. Arumugam, S., S. Schmieder, W. Pezeshkian, U. Becken, **C. Wunder**, D. Chinnapen, J.H. Ipsen, A.K. Kenworthy, W. Lencer, S. Mayor, and **L. Johannes**. 2021. Ceramide structure dictates glycosphingolipid nanodomain assembly and function. ***Nat. Commun.*** 12:3675.

4. Forrester, A., S.J. Rathjen, M.D. Garcia Castillo, C. Bachert, A. Couhert, L. Tepshi, S. Pichard, J. Martinez, H.-F. Renard, C.A. Valades Cruz, F. Dingli, D. Loew, C. Lamaze, J.C. Cintrat, A.D. Linstedt, D. Gillet, J. Barbier, and **L. Johannes**. 2020. Functional dissection of the retrograde Shiga toxin trafficking inhibitor Retro-2. ***Nat. Chem. Biol***. 16:327–336.

5. Watkins, E.B., J. Majewski, E.Y. Chi, H. Gao, J.C. Florent, and **L. Johannes**. 2019. Shiga toxin induces lipid compression: a mechanism for generating membrane curvature. ***Nano Lett.*** 19:7365-7369.

Expected profile of the candidate

Candidates should have experience or be able to learn within a short period of time in as many of the following techniques as possible: cell-based assays, molecular biology (cloning), confocal microscopy, electron microscopy, data analysis using bioinformatics tools, recombinant protein expression and purification (HPLC/FPLC).
Previous experience with CRISPR/Cas9 gene editing and organoid preparation would be an advantage.
The candidate should be highly motivated, fluent in English, and have excellent interpersonal and communication skills to collaborate with different teams.