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EuReCa International PhD Program

PhD thesis project

2020 Call for application

Molecular architecture of endoplasmic reticulum-plasma membrane contact site by cryo-electron tomography

General information

Call	2020
Reference	2020-04-LEVY
Keyword(s)	Membrane contact site cryo-electron microscopy interorganelle communication biochemistry image analysis

Director(s) and team

Thesis director(s)	Daniel Lévy & Manuela Dezi
Research team	Molecular Microscopy of Membranes
Research department	UMR 168 – Physico-Chimie Curie Lab

Description of the PhD thesis project

Our research aims at describing cell membranes and cytoskeletal elements at molecular level. We are interested in proteins involved in cell division, in cell detoxification and in communication between organelles. Our strategy relies on using in vitro systems to identify minimal functional units and modulate parameters that are difficult to access in cell. We use cryo-EM to get 3D architectures of both proteins and membranes at the highest resolution.

Cellular organelles communicate by intravesicular transport and trough membrane contact sites (MCS). MCS are involved in lipid homeostasis, Ca-signaling or organelle inheritance. MCS dysfunctions are reported in tumor progression and proteins are targets of anti-cancer drugs. There is no molecular model of MCS, which limits our understanding of the mechanisms of assembly/disassembly of MCS and associated functions. With cryo-electron tomography (cryo-ET) and in vitro reconstituted contact sites made of purified VAP-A in the ER and OSBP a lipid transporter on the Golgi, we have revealed the importance of intrinsically disordered regions of the constituent proteins in the 3D function and architecture of MCS (Jacmena D Dev Cell 2019, de la Mora in prep.).

PhD thesis will focus on Tricalbins3 in ER membrane and linked to Pip2 at the PM, probably involved in Ca-stimulated lipid transport. The goals are to determine the 3D architecture of Trcb3 from in vitro reconstituted MCSs at sub-nanometric resolution by cryo-ET, understand the molecular determinants of MCS assembly/disassembly, contextualize the in vitro results in a cellular environment, in collaboration with W. Kukulski (MRC, UK) who analyses Trcb3 by cryo-ET in cells.

This will be the first high resolution 3D model of proteins engaged in MCS with expected large impact on the understanding of MCS in general. The project uses approaches of membrane biochemistry, cryo-EM, image analysis, structural biology and cell biology.

International, interdisciplinary & intersectoral aspects of the project

The Team Molecular Microscopy of Membrane is composed of biophysicists and biologists. The project uses approaches of membrane biochemistry, cryo-EM, image analysis and structural biology to understand a major cell function. It involves concepts of in vitro design and reconstitution of minimal machinery to reproduce a function, membrane biophysics for membrane remodeling associated with MCS and computational signal analysis for cryo-tomogram processing. Through our collaboration with W. Kukulski (LMB, MRC, UK), it combines multi-scale approaches from molecular to cellular. Through interaction with DIVA, the project will extend to approaches of deep learning and augmented reality.

Recent publications

1. Jamecna D, Polidori DJ, Mesmin B, **Dezi M**, **Lévy D**, Bigay J, Antonny B (2019). An intrinsically disordered region in OSBP acts as an entropic barrier to control protein dynamics and orientation at membrane contact sites Developmental cell: * highlighted Trend in Cell Biology 2019: DOI: 10.1016/j.devcel.2019.02.021.

2. Simon C*, Kusters R*, Caorsi V*, Allard A, Abou-Ghali M, Manzi J, Di Cicco A, **Lévy D**, Lenz M, Joanny J-F, Campillo C, Plastino J, Sens P*, Sykes C* (2019) Actin dynamics drive cell-like membrane deformation. Nature Physics: DOI: 10.1038/s41567-019-0464-1.

3. Beber A, Taveneau C, Nania M, Tsai FC, Di Cicco A, Bassereau P, **Lévy D**, Cabral JT, Isambert H, Mangenot S*, Bertin A* (2019). Membrane reshaping by micrometric curvature sensitive septin filaments Nature communications: DOI: 10.1038/s41467-019-08344-5.

4. P Guichard, V Hamel, M Le Guennec, N Banterle, I Iacovache, V Nemčíková, I Flückiger, K N Goldie, H Stahlberg, **D Lévy**, B Zuber, P Gönczy (2017) Cell-free reconstitution reveals centriole cartwheel assembly mechanisms. Nature communications: 14813: DOI: 10.1038/ncomms14813.

5. Guillaume van Niel, Ptissam Bergam, Aurelie Di Cicco, Ilse Hurbain, Alessandra Lo Cicero, Florent Dingli, Roberta Palmulli, Cecile Fort, Marie Claude Potier, Leon J Schurgers, Damarys Loew, **Daniel Levy**, Graça Raposo (2014) Apolipoprotein E Regulates Amyloid Formation within Endosomes of Pigment Cells. Cell reports: 43-51: DOI: 10.1016/j.celrep.2015.08.057.

Expected profile of the candidate

Applicants should have a strong desire to explore cell biological phenomena in an in vitro context, an interest in cryo-EM and computational analysis and should show solid capacity for independent and creative thinking. Background in biophysics or structural biology is strongly recommended. During the PhD thesis, the student will be trained in the membrane biochemistry, cryo-electron microscopy and image analysis with the help of biochemists and cryo-electron microscopists in the Molecular Microscopy of Membranes group.