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

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Host laboratory:
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Host group/team:
MICA and ISBG

Title of the M2 research internship:
Illuminating viral Infection by developing Correlative Light and Electron Microscopy approaches

Project summary:
Respiratory syncytial virus (RSV) is the leading cause of child bronchiolitis and pneumonia, with a burden on the elderly comparable to influenza. Despite recent prophylactic advances, no effective therapeutic treatment exists yet. To establish and spread infection, the virus replicates its genome within host cells and transmits it to neighbouring cells. Our research aims to unravel the spatio-temporal and mechanistic details of these processes.
Alongside structural and biochemical studies with purified viral proteins and complexes, observing the virus directly inside the cell at different stages of transcription, replication, and assembly is key. This can be achieved by combining two very powerful and complementary imaging modalities: fluorescence and electron microscopy (FM and EM). Correlative light and electron microscopy (CLEM) should allow us to locate the desired events within cells using a specific fluorescent protein as a label fused to one of the viral proteins. First, as only fluorescently labelled objects are visible in FM images, they can be easily located; then, the identified coordinates can be used to target EM imaging precisely to these regions of interest. This can be done at room temperature, on sections of resin-embedded cells, to reveal the cellular context and morphology of viral intermediates, or under cryogenic conditions to preserve the native state of the cell, enabling high-resolution 3D imaging of these intermediates and their supramolecular assemblies. Instead of using an infectious virus, we propose employing transfection systems with plasmid-expressed viral proteins, allowing for easier mutational analyses and optimisation of fluorescent protein fusions.
This M2 project, at the interface of imaging technology and virology, aims to establish room-temperature CLEM as a working pipeline and evaluate the efficiency of different fluorescent protein fusions under cryogenic conditions. Beyond advancing the CLEM setup for the IBS community, the student will address important virology questions by characterising the cellular context, morphology, and ultrastructure of RSV transcription and replication factories in the cell cytoplasm, and visualising viral assembly intermediates at the cell membrane. The M2 project is a launchpad for a PhD for those eager to push imaging technologies forward while tackling fundamental virology challenges – the envisioned PhD would extend into cryo-super resolution fluorescence imaging and cryo-CLEM to guide cell thinning by cryo-focused ion beam milling followed by high resolution cryo-electron tomography analysis of different intermediates of RSV infection. Ultimately, this work will contribute towards realising dynamic 3D structural movies of the infection process.

Keywords:
Fluorescence microscopy, electron microscopy, correlative imaging, respiratory syncytial virus, RSV

Relevant publications of the team:
Structural landscape of the respiratory syncytial virus nucleocapsids. Gonnin L, Desfosses A, Bacia-Verloop M, Chevret D, Galloux M, Éléouët JF, Gutsche I. Nat Commun. 2023 Sep 15;14(1):5732. doi: 10.1038/s41467-023-41439-8. PMID: 37714861

Photophysical Studies at Cryogenic Temperature Reveal a Novel Photoswitching Mechanism of rsEGFP2. Mantovanelli AMR, Glushonkov O, Adam V, Wulffelé J, Thédié D, Byrdin M, Gregor I, Nevskyi O, Enderlein J, Bourgeois D. J Am Chem Soc. 2023 Jul 12;145(27):14636-14646. doi: 10.1021/jacs.3c01500. PMID: 37389576