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Topic of the project: In situ characterisation of viral replication factories

Responsible PIs:

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Short project description (max. 500 words)*
To support their replication after infecting a eukaryotic cell, positive-sense single strand RNA (+ssRNA) viruses induce a dramatic remodelling of host cell intracellular membranes to form one of two distinct structures: first, spherules corresponding to membrane invaginations as exemplified by dengue virus (DV) and zika virus; second, double-membrane vesicles (DMVs) corresponding to membrane exvaginations that resemble autophagosomes as exemplified by hepatitis C virus (HCV) and SARS-CoV-2. Functionally, these virus-induced membranous structures, referred to as viral replication factories or replication organelles (ROs), provide specialised compartments conducive to efficient viral genome amplification while at the same time shielding the viral RNA against hostile cellular attacks. Our current understanding of the biogenesis, the molecular composition, the activity, and the structural properties of these two RO types is very limited. Here, we propose an interdisciplinary project at the intersection of molecular virology and structural biology aimed at providing a comprehensive functional and structural characterisation of these two RO morphotypes. Building on our profound expertise in viral and host cell factors involved in RO biogenesis, in this project we will investigate the spatial and structural organisation of the two RO morphotypes, as induced by DV and HCV, within the native environment of infected host cells by combining cryogenic light and electron microscopy (cryo-CLEM) with *in situ* cryogenic electron tomography (cryo-ET). The recent advances of cellular structural biology methods will enable us to study at high-resolution the structure and the interaction network of both the viral and the host protein factors involved in the formation and activity of viral ROs. Obtained data will be functionally validated by using well-established cell biology and molecular virology assays. By investigating different +ssRNA viruses inducing the two distinct RO morphotypes, we also aim at understanding the conserved and distinct mechanisms underlying this fundamental viral process while, at the same time, elucidating the specific adaptation mechanisms that evolved with these diverse virus genera.

Research Group description 1 (max. 250 words)*
The Bartenschlager group studies +ssRNA viruses. A particular focus is put onto the biogenesis, architecture and functionality of the replication organelles of these viruses with the final goal to exploit gained knowledge for the development of (broad-spectrum) antiviral drugs. To reach our ambitious goals, we employ various -omics based approaches, combined with high-content phenotyping screening (e.g., Tabata et al., 2021). Another

important tool we are using are imaging-based approaches to determine the 3D organization of viral ROs and how they are linked to the cellular endomembrane system (e.g., Cortese et al., 2020). This includes electron tomography and correlative imaging approaches (e.g., Lee et al., 2019). In this way, we are able to combine morphological and functional data to understand how viral proteins trigger host cell membrane rearrangements, to identify involved viral and host cell factors (proteins and lipids) and to use this knowledge to identify targets for antiviral therapy, ideally having broad-spectrum activity.

Research Group description 2 (max. 250 words)*

Simone Mattei is Team Leader for Electron Microscopy Service and Technology Development at the EMBL Imaging Centre, a new service unit providing the international scientific community with a broad spectrum of cutting-edge light and electron microscopy technologies to study macromolecular, cellular, and full organism systems across the scales of biology. Our research focuses on the development and implementation of advanced imaging methods, including cryogenic correlative light and electron microscopy (cryo-CLEM) workflows. Our interdisciplinary team is composed of engineers, software developers, image analysts, and molecular biologists working together to improve the achievable resolution in both imaging modalities while at the same time optimising the reproducibility and the robustness of the methods (e.g., Weis et al., 2021). We combine the hardware and software development with the implementation of machine learning approaches for automated and intelligent targeting of the region of interest within the native cellular context. By combining the strengths of the individual imaging modalities, the correlative approaches allow us to bridge the temporal and compositional information of fluorescence microscopy with the high-resolution structural information of electron microscopy. The developed methods are tested and applied on multiple viral and eukaryotic model organisms to study fundamental biological processes by means of *in situ* cellular structural biology. We are interested in the functional and structural characterisation of macromolecular machineries involved in the editing of mitochondrial mRNA, the maturation of mitochondrial ribosomes, the co-translational targeting of membrane proteins, and the life cycle of enveloped viruses (e.g., Mattei et al., 2016; Mattei et al., 2018).

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