The apoptotic factor Apaf1 inside cells during cell death - a case for cryo-CLEM

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The cell death program known as apoptosis is essential for organisms to remove surplus cells. This mechanism is important during development as well as for everyday tissue maintenance. Apoptosis also prevents uncontrolled proliferation of potentially cancerous cells. During the program, cytochrome c is released from mitochondria where it interacts with Apaf1 in the cytosol. This is known as the formation of the apoptosome complex, a key event in apoptosis. This complex serves as a platform for the activation of caspases that execute the dismantling of the cell. We have found that the inside cells, the apoptosome is a pleomorphic, continuous meshwork structure that forms transiently during cell death [1]. In this talk, I will present our work towards understanding the *in situ* apoptosome assembly.

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RESOLVING ARP2/3 COMPLEX-MEDIATED BRANCH NUCLEATION IN SPACE AND TIME USING CRYO-ELECTRON TOMOGRAPHY

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In this talk, I will demonstrate the potential of cryo-electron tomography (cryo-ET) for time-resolved *in situ* structural biology research. To illustrate this, I will use our ongoing study of native actin assemblies. Actin plays a pivotal role in numerous cellular processes by forming and disassembling highly dynamic, ordered structures. Nevertheless, our understanding of how the molecular components of the actin machinery collaborate within cells to produce nanonewton force-generating actin systems, such as podosomes in human macrophages or those involved in endocytosis in yeast, remains limited. I will demonstrate how cryo-ET can be employed to reveal the polarity of actin filaments at the monomer level within podosomes in human macrophages. This approach provides valuable insights into podosome assembly dynamics. It can also be used to recreate the spatial and temporal distribution of Arp2/3 complex-mediated branch junctions in both human macrophage podosomes and in yeast during clathrin-mediated endocytosis. To achieve this, we analyzed the spatial orientation of branch junctions in our cellular tomograms using high-resolution template matching and subtomogram averaging. Based on this information, we simulated successive generations of actin filament branches. We validated our model predictions by comparing the generations assigned by our model with those visualized in our data. Finally, I will introduce CryoAll: a Vision Transformer model trained on cryo-ET data that can segment various cellular structures, including cytoskeletal elements. Classical methods would fail to do this due to the density and complexity of these actin networks. Our integrative approach therefore reveals the assembly mechanisms of native actin networks at the molecular level, helping us to understand the underlying force generation mechanisms. Proposing time-resolved *in situ* cryo-electron tomography, this work opens up new avenues in the field of 4D structural biology.

High-resolution Cryo-EM of P-gp and its regulation through lipids and susbtrates

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ATP-binding cassette (ABC) are a large group of primary active transporters present in the whole kingdom of life. They use the energy of ATP binding, hydrolysis, and ADP/P_i release to pump a wide and diverse array of substrates across the membrane and are well known as lipid-floppases or as polyspecific multidrug efflux pumps with major implications in the clinic. ABC transporters are highly dynamic, undergoing large-scale conformational changes for substrate translocation. Their activity is regulated by the presence of substrates, but especially through the intricate interplay between the membrane protein and its surrounding lipid environment.
The underlying molecular mechanisms of this stimulation remain poorly understood. Likewise, the actual time scales of the conformational changes required for substrate transport are unknown. Moreover, it is impossible to resemble an accurate representation of the plasma membrane when synthetic hydrophobic environments, e.g., nanodiscs, polymers, or detergents, are used, fundamentally challenging experiments addressing interactions between lipids and proteins.

The ABC transporter P-glycoprotein (p-gp) is a human efflux pump, and high expression levels have been identified in the small intestine, liver, kidneys, and blood-brain barrier, promoting rapid detoxification of absorption-sensitive or absorption-restricted tissues. P-gp exports many amphiphilic, as well as neutral or cationic compounds, which are structurally unrelated. This polyspecificity reduces drug absorption and limits their effective clinical use. P-gp overexpression in cancer cells is linked to the onset of MDR, allowing tumor cells to export and thus evade many established chemotherapeutics.

In my talk, I will present various approaches and structural data that illustrate the symbiosis between human P-glycoprotein and the membrane and highlight how these proteins can distinguish between environmental and substrate stimulation, revealing the regulation of these fundamental molecular machines.

Large-scale 3D imaging of cellular actin networks using cryo-ET

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Dynamic cellular behaviors—such as cell motility and shape adaptation—are driven by protrusive forces generated by the actin cytoskeleton. Actin filaments assemble into higher-order networks with diverse physical properties, shaped by the interplay between actin filament biochemistry and network architecture.

Cryo-electron tomography (cryo-ET) has become a powerful tool for visualizing the organization of these actin networks in situ. However, traditional cryo-ET methods lack the temporal resolution and large-scale imaging context needed to fully capture the structural and functional versatility of these complex systems.

We develop novel workflows that integrate optimized specimen preparation, montage cryo-ET data acquisition and image processing to enable large-scale, molecular-resolution imaging of cellular regions such as the leading edge—the dynamic front of motile cells. This approach provides a ground-truth framework for understanding how the geometrical complexity of the actin cytoskeleton guides directional cell migration.

By combining our high-resolution cryo-ET workflows with biochemical and cell biological analyses, we resolve the structures of both known and previously uncharacterized actin-binding proteins. This allows us to localize these proteins within their native cellular architectures and link molecular structure to higher-order organization and function. Together, these advances offer a platform for uncovering the structural underpinnings of emergent cellular behaviors dependent on the actin cytoskeleton.

DECODING RESPIRATORY SYNCYTIAL VIRUS REPLICATION: IN SERENDIPITY WE TRUST

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Respiratory syncytial virus (RSV) is the leading cause of child bronchiolitis and pneumonia, with a burden on the elderly comparable to that of influenza. Although prophylactic options such as vaccines and monoclonal antibodies have recently become available, no direct-acting RSV antiviral exists to date. Replication and transcription of RSV genetic material are critical steps for establishing and spreading infection, making them attractive therapeutic targets. These functions take place inside cytoplasmic membranelles biocondensates called viral factories and are carried out by the viral ribonucleoprotein particles, composed of the viral genomic RNA, which is enwrapped by the viral nucleoprotein into a helical nucleocapsid, and the RNA-dependent RNA polymerase complex. In this talk, we will dive into the structural landscape of the RSV nucleocapsids and explore the molecular mechanisms and extent of their polymorphism using single particle cryo-EM. We will also see how a single mutation that mimics a post-translational modification of the nucleocapsid observed in RSV infected cells dramatically alters nucleocapsid structure and function. In addition, we will have a first glimpse into the structures and the spatial organisation of the nucleocapsids within the viral factories of RSV-infected cells using cryo-electron tomography. This ongoing work represents a new step towards elucidating the structural basis and functional implications of the non-canonical helical symmetry of RSV nucleocapsids and provides a foundation for investigating dynamic conformational changes of the RSV transcription and replication machinery in action. Nevertheless, each new structure raises more questions than it answers, underscoring how much remains to be discovered about the molecular mechanisms of RSV genome synthesis. This work is a collaborative effort between three French research teams: MICA (IBS Grenoble), BMP (VIM Jouy-en-Josas), and MMP (Institut Pasteur).

IMAGING VIRAL GLYCOPROTEINS INVOLVED IN VIRUS ASSEMBLY AND CELL ENTRY

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This presentation describes cryogenic electron microscopy (cryo-EM) experiments aimed at understanding how lipid-enveloped viruses such as influenza viruses or retroviruses assemble and how they enter cells by membrane fusion. During influenza A virus entry, the haemagglutinin (HA) glycoprotein binds cell surface receptors and mediates membrane fusion with the endosome membrane. We apply single particle microscopy to identify structural changes in the uncleaved HA precursor (HA0) at low pH to inform structural transitions in HA important to membrane fusion and to understand the role of the cleavage of the precursor in the fusion activity [1]. In addition, we apply electron cryotomography to study the structural basis of lattice assembly by the single surface glycoprotein (HEF) of influenza C virus [2,3]. Foamy viruses, are an ancient subfamily of retroviruses. By combining cryo-EM and electron cryotomography, we determined structures of the foamy virus particle including the envelope glycoprotein (Env) and capsid *in situ*. The structure of Env is different from other retroviral glycoproteins but is related to the F glycoprotein in paramyxoviruses and the spike glycoprotein (S) of coronaviruses. The capsid is icosahedral but with features of pleomorphic orthoretroviral capsids. The integrated structure of foamy virus informs on the roles of the glycoprotein and capsid in virus assembly [4]. Our integrated studies of viral envelopes is relevant to understanding a wide range of protein assemblies on membranes.

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