Invited Speaker

485 Viral replication organelles revisited by cryo-electron microscopy
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Oral Presentation

67 CoCID: Compact Cell Imaging Device for Correlative Investigation of Hepatitis E Infection
Dr. Christopher Evans[1], Dr. Kenneth Fahy[2], Dr. Sergey Kapishnikov[2,3], Mrs. Tiina O'neill[4], Dr. Dimitri Scholz[4], Ass. Prof. Nicola Fletcher[1,4]
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135 Secondary envelopment of the human cytomegalovirus is a fast process, utilizing the endocytic compartment
Tim Bergner[1], Laura Cortez Rayas[2], Jens von Einem[2], Dr. Clarissa Read (Villinger)[1]
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492 Label-free imaging of virus-cell interactions using 200 Hz ROCS microscopy
Dominik Huber[1], Prof. Dr. Alexander Rohrbach[1]
[1]Lab for Bio- and Nano-Photonics, University of Freiburg, Freiburg, Germany

505 Ultrastructural investigation of human cytomegalovirus tegument protein UL71 and its role in secondary envelopment
Benedikt Küß[1], Annika Metzner[2], M.Sc. Laura Cortez Rayas[2], Prof. Dr. Paul Walther[1], Dr. Gregor Neusser[3], apl. Prof. Dr. Christine Kranz[3], Dr. Clarissa Read[1,2], apl. Prof. Dr. Jens von Einem[2]
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511 Advanced imaging reveals new lipid droplets dynamics in the malaria parasite Plasmodium falciparum
Jiwon Lee[1,2], Professor Kai Matuschewski[3], Associate Professor Giel van Dooren[2], Professor Alexander G. Maier[2], Assoc. Prof. Melanie Rug[1]
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544 Correlative cryo-bioimaging to study coronavirus replication organelles
Mr Patrick Phillips[1,2,3], Prof Philippa Hawes[4], Prof Maria Harkiolaki[2], Dr Dan Clare[2], Prof Jonathan Grimes[3], Dr Helena Maier[1]
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Poster Presentation

168 Structure of the first isolated polinton-like virus and its host
Dr. Gabriela N. Condezo[1], Mr. Roberto Marabini[2], Mr. Javier Conesa[1], Mr. David Delgado[1], Mrs. Hilde K. Stabell[3], Mr. Gunnar Bratbak[3], Mrs. Carmen San Martín[1]

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477 Visualizing microbial interactions and CRISPR-Cas interference using FISH applied to environmental archaeal biofilms

Indra Banas[1], Dr Sarah P. Esser[1], Dr. Cristina Moraru[1], Prof. Dr. Alexander J. Probst[1,2,3]
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482 Interaction of transduction enhancing peptide nanofibrils with cells and virions assessed by complementary EM techniques

Julia La Roche[1], Lena Wirth-Rauch[2], Laura Zimmermann[2], Dr. Kübra Kaygisiz[3], Prof. Dr. Tanja Weil[3], Prof. Dr. Jan Münch[2,4], Dr. Clarissa Read[1]
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508 Structural basis of rotavirus spike proteolytic activation

Dr Dunia Asensio-Cob[1], Dr Carlos Perez-Mata[2], Dr Josue Gomez-Blanco[3], Dr Javier Vargas[3], Dr Javier M Rodriguez[2], Dr Daniel Luque Buzo[4]
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966 Elimination of HCV replication machinery early after antiviral treatment with DAA monitored by multimodal microscopy

Dr. Victoria Castro[1], Gema Calvo[1], Ana Pérez-Berna[2], David Rogers[3], Stephen O'Connor[3], Sergey Kapishnikov[3], Paul Sheridan[3], Eva Pereiro[2], Kenneth Fahy[3], Dr. Pablo Gastaminza[1]
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1111 Synthetic Bispecific RBD Antibody Effectively Neutralizes SRAS-CoV-2

Uijin Kim[1], Dong-Gun Kim[2], Inho Park[3], Youngki Yoo[1], Bumhan Ryu[4], Ga-Yeon Yoon[1], Kitaek Nam[3], Jeonsoo Shin[3], Hak-Sung Kim[1], Hyun-Soo Cho[1]
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Late Poster Presentation

1265 Investigating VSV-GP replication using TEM & STEM tomography of high pressure frozen, freeze substituted samples

Johannes Georg Wieland[1,2], Rebecca Habisch[1], Dr. Eva Puschmann[1], Dr. Mareike Elvert[1], Prof. Dr. Paul Walther[2], Dr. Jorge Soza Ried[1], Dr. Martin Dass[1]
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Viral replication organelles revisited by cryo-electron microscopy

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Coronaviruses and their distantly related arteriviruses belong to the order Nidovirales. Similar to other positive-strand RNA viruses infecting eukaryotes, nidoviruses hijack host membranes converting them into specialized structures often termed viral replication organelles. These replication organelles exhibit diverse morphologies depending on the virus group but serve a common purpose: to establish specialized micro-environments conducive to viral RNA synthesis, potentially shielding viral replication intermediates from cellular immune surveillance. A hallmark of nidovirus infection is the emergence of double-membrane vesicles (DMVs) originating from endoplasmic reticulum membranes, which serve as sites of viral RNA synthesis. Typically, nidovirus genomes encode 3 nonstructural proteins (nsps) that contain multi-spanning transmembrane (TM) domains (TM1, TM2 and TM3) and that are the main drivers of DMV formation (1).

Traditionally, viral replication organelles have been studied by conventional electron microscopy (EM) methods on resin-embedded infected cells. However, recent advancements in cryo-EM, particularly the introduction of focused ion beam (FIB) milling technology that makes internal regions of cells accessible for cryotomography, have revolutionized our ability to investigate these virus-induced structures. This cutting-edge approach allowed us to investigate viral replication organelles in cells infected with coronaviruses with exquisite detail (2). Through this methodology, we uncovered a molecular pore spanning the membranes of the DMVs, potentially serving as a gateway for viral RNA synthesized within the DMVs to access the cytosol for translation or encapsidation into progeny virions. Using subtomogram averaging, this crown-shaped pore complex of around 3 MDa was solved to 3 nm resolution and revealed to have an overall 6-fold symmetry. Additionally, we identified that the largest viral nonstructural protein, which contains the TM1 domain, as a component of this complex. Furthermore, in situ cryo-tomograms provided compelling evidence of dynamic interactions between the pore complex and other macromolecules on both sides of the pore. Based on these observations, we postulated that the viral replication machinery may interact with the pore side facing the DMV interior, while the nucleocapsid protein, responsible for encapsulating new viral RNA, would associate with it on the cytosolic side.

Our investigations have extended to the distantly related arteriviruses, revealing the presence of similar DMV-spanning pore complexes. Notably, viral nucleocapsids and putative ribonucleoprotein assemblies were often observed in close association with the arteriviral DMV pores, providing further support to the notion of a functional linkage between RNA export from the DMVs and encapsidation, mediated by interactions of the pore complex with the nucleocapsid protein. Interestingly, DMV pore complexes were also formed in non-infected cells that expressed the 3 arterivirus TM-containing nsps. This indicated that the formation of a DMV-spanning pore structure is independent of the presence of viral RNA, the replication machinery, or the structural proteins. Moreover, expression of only the nsps containing TM1 and TM2 was sufficient to form DMV-like structures in which pore complexes were present. This observation is consistent with recent reports of the formation of DMV-spanning pore complexes in cells expressing coronavirus TM1- and TM2-containing nsps (3). Taken together, these results suggest that DMV-spanning pore complexes may be a shared characteristic among nidoviruses. These pore complexes would have a similar mechanism of formation driven by

TM1- and TM2-nsps, which appear to be core components of the complex, and would fulfill a critical role in coordinating viral RNA synthesis, export and encapsidation.
Interestingly, recent studies focusing on other +RNA viruses that induce a morphologically different type of viral replication organelle consisting of an invaginated spherule, have unveiled crown-shaped multimeric complexes located in the neck of the spherules (4,5). These complexes bear intriguing similarities to the DMV-spanning complexes observed in nidoviruses. Collectively, these studies define an emerging class of viral complex that seem to underscore common strategies employed in the replication of evolutionarily distant positive-strand RNA viruses. Further elucidation of their structural and functional details will not only deepen our understanding of viral replication but may pave the way for the development of novel antiviral strategies.

Keywords:

coronaviruses, arteriviruses, cellular cryotomography

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CoCID: Compact Cell Imaging Device for Correlative Investigation of Hepatitis E Infection

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background: Hepatitis E virus (HEV) is an burgeoning, zoonotic viral infection, and is the leading cause of acute hepatitis worldwide [1]. However, the lack of robust in vitro models supporting HEV infection [2] has hindered comprehensive studies on the various stages of viral infection and its impact on host cells [3].
Methods: CoCID [4] employs novel lab-scale Soft X-ray Microscopy (SXM) [5], a technology currently limited to select synchrotron facilities worldwide, enabling rapid, label-free imaging of whole, hydrated cells. We utilized model infection systems, including HEV genotype 1 and 3 replicon-transfected human hepatocytes, to investigate structural changes during infection. Additionally, we examined large, scaffold-free, polarized 3D spheroid models infected with clinical HEV to compare these pathological changes during full virus infection.
Results: Analysis of HEV replicon-transfected cells using SXM revealed a statistically significant increase in mitochondrial elongation. This observation was corroborated by light and electron microscopy techniques. Furthermore, examination of 3D spheroid models infected with clinical HEV demonstrated notable structural changes, which were explored using Correlative Electron-Soft X-ray Microscopy (CEXM) and Correlative Light-Electron microscopy (CLEM).
Conclusions: Our study demonstrates the utility of SXM and correlative approaches in elucidating the dynamics of HEV infection. The observed structural changes in infected cells provide further understanding of HEV biology and may inform the development of targeted therapeutic interventions against HEV. Moreover, the development of a Correlative Light-Electron-Soft X-ray Microscopy (CLEXM) workflow enhances our capability to investigate viral pathogenesis.

Keywords:

Hepatitis-E, Virus, Soft-Xray, Correlative, Microscopy

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Secondary envelopment of the human cytomegalovirus is a fast process, utilizing the endocytic compartment

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background and aims: The human cytomegalovirus (HCMV), a member of the herpesvirus family, is an enveloped virus. It gains its final envelope by the critical but not well understood secondary envelopment process that takes place at the cytoplasmic viral assembly complex (cVAC). Partially tegumented capsids that were released from the nucleus and transported to the cVAC initiate a budding process into cellular membranes coated with viral glycoproteins. Previous studies present controversial results regarding the origin of the viral envelope, suggesting both, trans-Golgi and endosomal origin, as well as a similarity with exosomes (1-4). Thus, the identity of the membranes utilized for secondary envelopment remains unclear. This study investigated the role of endocytic membranes for secondary envelopment of HCMV.
Methods: Wheat germ agglutinin (WGA) either conjugated to a fluorophore or to horseradish peroxidase (HRP) were used for labelling of glycoproteins at the plasma membrane of HCMV infected cells. By this, endocytosed membrane could be visualized by fluorescence and electron microscopy (EM). Membrane marker screening was used to narrow down the potential origin of the membrane compartment used for cVAC formation and secondary envelopment. EM was used to directly visualize endocytosed membranes, HCMV capsids and their interactions. For EM, we adapted a WGA-HRP labeling protocol followed by the DAB reaction in living cells (5). Subsequently, EM samples were prepared by high-pressure freezing and freeze substitution for optimal membrane visibility and analyzed by quantitative transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) tomography.
Results: WGA was taken up into different membrane compartments within the cVAC of HCMV infected cells, including early endosomes, multivesicular bodies, trans-Golgi and recycling endosomes. TEM analysis showed budding of capsids at WGA-positive membranes and numerous enveloped WGA-positive capsids at the cVAC. Almost 90% of all budding capsids and 50% of enveloped capsids were labelled with WGA within 90 min. This indicated a rapid turnover of membranes at the cVAC in which freshly endocytosed membranes were transported from the plasma membrane into the cVAC to be utilized by capsids for their envelopment and consequent release into the extracellular space. Furthermore, we could show that 30 min are sufficient to complete endocytosis from the plasma membrane and secondary envelopment at the cVAC. STEM tomography revealed budding of capsids at various membranes, including trans-sided Golgi cisternae.
Conclusion: The endocytic compartment serves as the major membrane source for the formation of the cVAC as an optimized environment for virion maturation and consequently, as the major membrane source for secondary envelopment of HCMV. Furthermore, we show that secondary envelopment of HCMV is a fast process, in which endocytosed membranes are transported from the plasma membrane to the cVAC within minutes to be utilized for envelopment.

Keywords:

WGA-HRP, in-vivo DAB-labelling, TEM, STEM

Reference:

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5 Ellinger et al. (2010). doi: 10.1016/j.jsb.2009.10.011

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Label-free imaging of virus-cell interactions using 200 Hz ROCS microscopy

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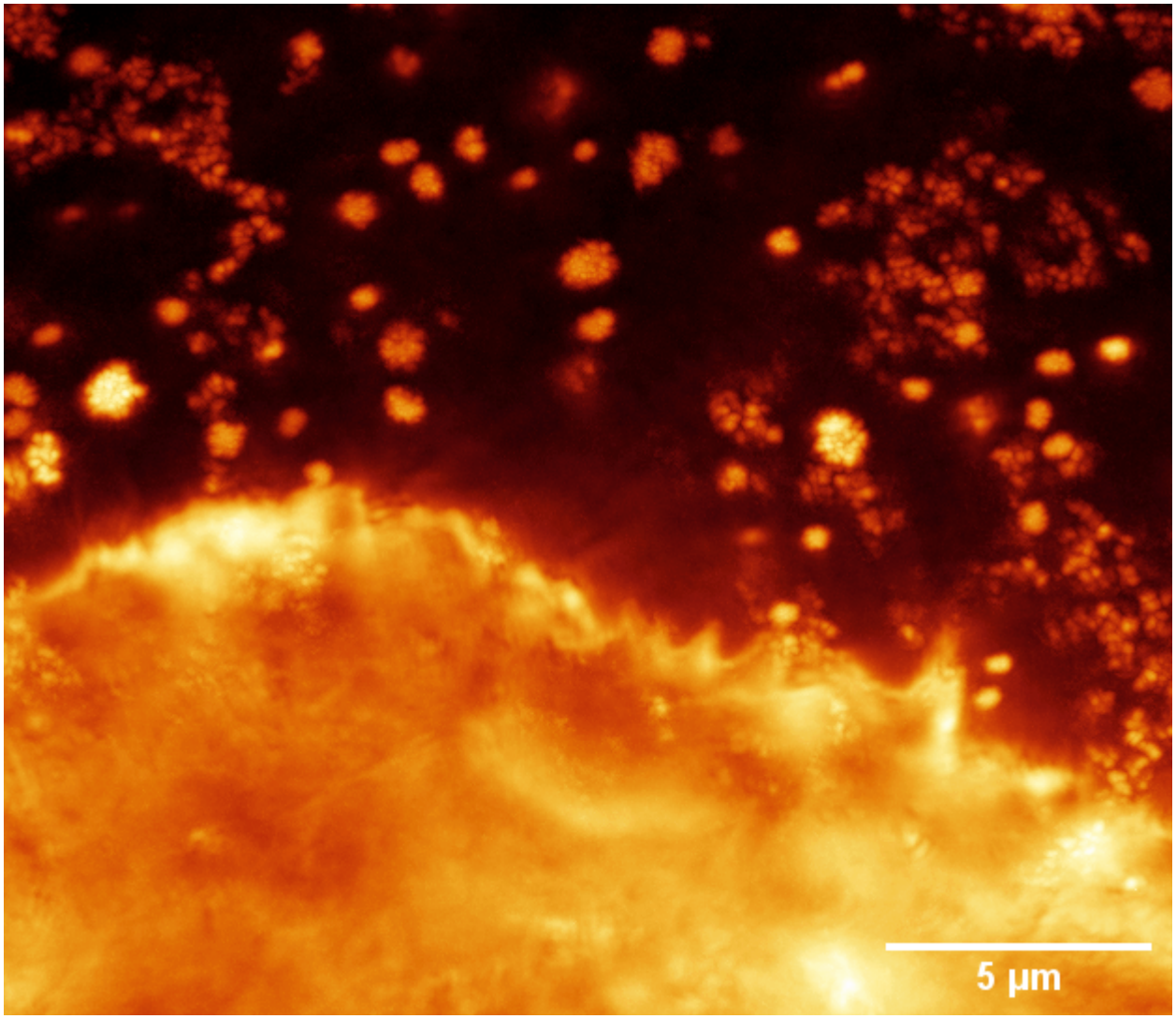
LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background incl. aims
The emergence of SARS-CoV-2 has shown the profound impact that viruses can have on human health and society. Due to their small size and remarkable speed it is very challenging to observe cell-virus interactions in life-cell imaging. Techniques like fluorescence microscopy, often struggle to visualize these interactions, especially due to their susceptibility to bleaching and the difficulty to label different structures without altering their function. This underscores the ongoing need for innovative approaches to unravel the complexities of virus-cell interactions and advance our understanding of infectious diseases.
Methods
In our research we use Rotating Coherent Scattering (ROCS) microscopy in order to visualize the diffusion of 100 nm sized virus-mimicking particles and their interactions with macrophages or epithelial cells. Rotating Coherent Scattering (ROCS) microscopy, a label-free imaging technique, harnesses the coherent backscattering of a rotating laser beam for super-resolution (150nm). The method involves oblique illumination of the sample from all azimuthal directions, producing nearly artifact-free images by integrating all scattered light during a single laser rotation. Currently operating at a frame rate of 200 Hz, ROCS minimizes motion blur, without any need for extensive post-processing. With its capability to adjust the illumination angle up to 70° and to utilize 4 distinct illumination wavelengths in the range of 405 nm to 561 nm, ROCS proves highly versatile in brightfield or darkfield mode, transitioning between total internal reflection (TIR) and non-TIR.
Results
By tracking particle trajectories and analyzing their fluctuations we are able to analyze the speed of our diffusing virus-mimicking particles and are able to follow binding events of single particles to cells. After binding of particles to filopodia or lamellipodia the observed fluctuations decrease over time and Additionally, by using multiple illumination angles and wavelengths in ROCS microscopy we observe different appearances of differently absorbing structures, enabling the analysis of subtle z-motions of particles and the visualization of distinct cellular structures.
Conclusion
Our research highlights Rotating Coherent Scattering (ROCS) microscopy, as a powerful tool to observe virus-cell interactions. Through ROCS, we successfully image the diffusion of virus-mimicking particles and visualize discrete binding events to cells, enabling detailed analysis of particle trajectories and binding properties. Furthermore by capturing scattered light from multiple wavelengths at once, we extend the capability of ROCS towards obtaining specificity for different structures.



Keywords:

Rotating-Coherent-Scattering-Microscopy (ROCS), single-virus tracking, label-free

Reference:

Jünger, F., D. Ruh, D. Strobel, R. Michiels, D. Huber, … A. Rohrbach (2022). "100 Hz ROCS microscopy correlated with fluorescence reveals cellular dynamics on different spatiotemporal scales." Nature Communications 13(1): 1758.

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Ultrastructural investigation of human cytomegalovirus tegument protein UL71 and its role in secondary envelopment

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background
The molecular mechanisms underlying the assembly of infectious virions of human cytomegalovirus (HCMV), an important human pathogen associated with complications in immunocompromised patients and the leading cause of birth defects after congenital infection, are still not well understood (Griffith et al., 2015). An important step of HCMV morphogenesis en route to infectious virions is a cytoplasmic envelopment process, referred to as secondary envelopment. Tegument protein pUL71, which is highly conserved among herpesviruses, is known to play an important role in the envelopment process (Schauflinger et al., 2011). Several functional motifs, mainly located in the conserved N-terminus of pUL71, have been characterized in detail (Read et al., 2019; Metzner, 2022), whereas the role of the non-conserved C terminus of pUL71 is mainly unclear. However, a tetralysine motif in the C terminal region of pUL71 has revealed a mechanistic involvement of this region in HCMV morphogenesis (Read et al., 2019). Dissecting the functions of pUL71 during secondary envelopment brings us further to unravel the molecular mechanism of secondary envelopment and could lead to the identification of new antiviral targets.
Methods
The role of the C-terminus of pUL71 was investigated by generation and characterization of a mutant virus (TB71insHA-299) expressing a truncated version of pUL71 fused to an HA epitope lacking the last 61 amino acids with the tetralysine motif and the MIM2 domain. A first phenotypic characterization of TB71insHA-299 after infection of human fibroblasts was performed by immunofluorescence microscopy (IF). For direct visualization of secondary envelopment, transmission electron microscopy (TEM) was carried out on human fibroblasts infected with TB71insHA 299 that were fixated by high-pressure freezing and freeze substitution at five days post infection and embedded in epoxy resin. Stages of secondary envelopment were analysed on ultrathin sections by TEM and compared with cells that were infected with wild-type virus or a previously published virus mutant carrying a mutation in the C-terminal tetralysine motif of pUL71 (Read et al., 2019). In addition, 3D analysis was performed on these samples by focused ion beam scanning electron microscopy (FIB-SEM) and scanning transmission electron microscopy (STEM) tomography (Villinger et al., 2014).
Results
In IF experiments, TB71insHA-299 infected cells showed an accumulation of viral particles at the periphery of the cytoplasmic viral assembly complex (cVAC) at large circular structures which is similar to a mutant lacking pUL71. Quantification of secondary envelopment stages by EM analysis of TB71insHA-299 infected cells revealed an impaired secondary envelopment compared to the wild-type virus but no further defect compared to the well characterized virus with a mutation in the tetralysine motif at the C terminus of pUL71 (Read et al., 2019). Furthermore, it was shown that the accumulation of viral particles at the periphery of the cVAC at large circular structures in TB71insHA 299 infected cells were multivesicular bodies (MVB) with viral capsids attached to the whole

cytoplasmic face of the MVB membrane. Additionally, EM revealed abnormal budding of capsids at multiple budding sites (MBS). These ultrastructural differences and the impaired secondary envelopment are similar to a virus that is unable to express pUL71 (Schauflinger et al., 2011). In the virus with the mutated tetralysine motif, these ultrastructural differences could not be observed (Read et al., 2019).

Conclusion

In conclusion, the last 61 C terminal amino acids of pUL71, containing the tetralysine motif and especially the MIM2 domain of pUL71, seem to be of great importance for HCMV morphogenesis. This work demonstrates that additionally to the already characterized secondary envelopment defect caused by the mutation of the tetralysine motif, the other amino acids, most likely the MIM2 domain, carry additional functions for the formation of an intact cVAC and efficient secondary envelopment. This data argues for the controversially discussed involvement of the ESCRT machinery in HCMV morphogenesis and membrane remodelling.

Keywords:

HCMV, High-pressure freezing, virus morphogenesis

Reference:

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Advanced imaging reveals new lipid droplets dynamics in the malaria parasite Plasmodium falciparum

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LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Introduction
Plasmodium falciparum, the deadliest species of malaria parasites infecting humans, has a complex life cycle which requires transition between two intrinsically different hosts (human and mosquito). Hence, the parasite faces the challenges of having to navigate growth, proliferation, transmission, and sexual reproduction in vastly different host environments. Each of these cellular processes rely on coordinated lipid access and metabolism mechanisms, with the parasite either synthesising essential lipids de novo or acquiring them from its host environment in order to propagate. Lipid droplets (LDs) are organelles central to lipid and energy homeostasis across all eukaryotes but their roles and importance in P. falciparum are poorly understood.
We therefore set out to characterise the size, composition and dynamics of LDs across the disease causing intraerythrocytic stages of the parasite life cycle.

Methods
We applied a combination of advanced light microscopy techniques (split fluorescence emission analysis) and volume imaging using Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) in combination with inhibitors of essential enzymes in neutral lipid metabolism to characterise the dynamics and importance of LDs in the parasite's asexual life cycle stages.

Results
We observed significant LD changes in late schizont stages, suggesting a switch from lipid accumulation to lipid utilisation that precedes parasite egress from the host erythrocyte. We will furthermore report on observed connections between LDs and several other organelles, pointing to potential functional interactions. Employing inhibitors that specifically interfere with the synthesis or break-down of triacylglyerols (TAG), we found that LD function is essential for schizogony and counteracts lipid toxicity.

Conclusions
Our study of LDs across the asexual life cycle grants new insights into the dynamics of lipid synthesis, storage and utilisation in P. falciparum, which might provide a novel avenue into new intervention strategies to combat this devastating disease.

Keywords:

malaria, lipid droplets, 3D FIB-SEM

Reference:

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Correlative cryo-bioimaging to study coronavirus replication organelles

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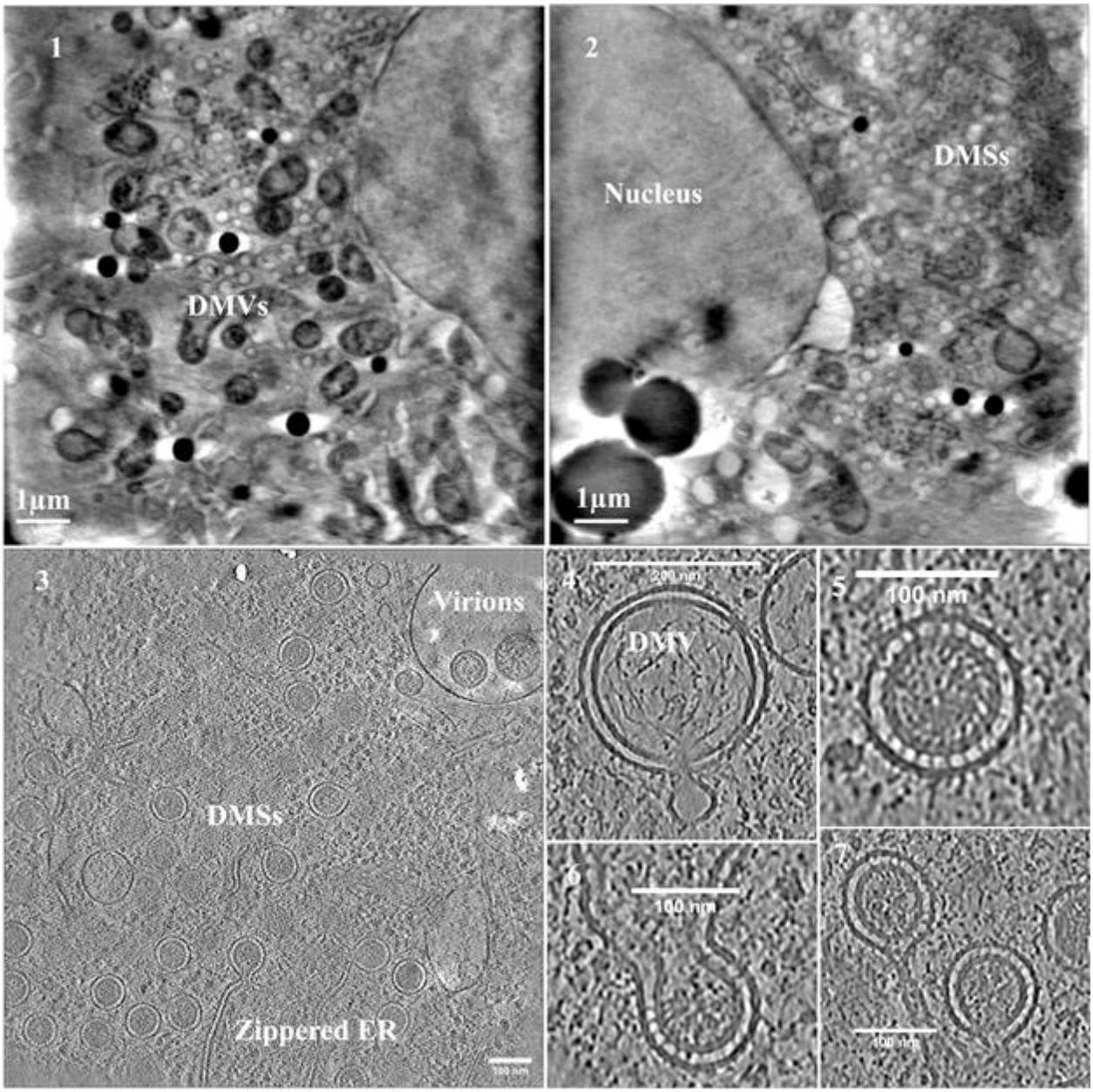
LS-06, Lecture Theater 4, august 30, 2024, 10:30 - 12:30

Background incl. aims
The Gammacoronavirus Infectious Bronchitis Virus (IBV) is a highly contagious pathogen of poultry and can be used as a model coronavirus (CoV) to study the formation and structure of CoV replication organelles (ROs). CoVs rearrange host cell membranes into ROs which act as platforms for the viral replication-transcription complex and are the sites of RNA genome replication. Conserved RO structures exist including double membrane vesicles (DMVs) and double membrane spherules (DMSs). The mechanisms of RO formation and the function of DMSs have not yet been elucidated. Using fluorescent recombinant viruses and correlative cryo-bioimaging, ROs will be marked and directly targeted for imaging using X-rays and electrons. These techniques will reveal RO structures over multiple scales, in 3D, to high resolution, and under near-native conditions. The aims are to develop a correlative workflow to observe the RO network in whole cells, RO formation events, and DMSs under cryogenic conditions. Subvolume averaging of electron tomograms will reveal membrane associated proteins which may give clues to RO formation mechanisms and functions.
Methods
Fluorescent tags were inserted at the N-terminus of nonstructural protein 2 (nsp2) using reverse genetics. The growth kinetics of recombinant IBVs were characterised in comparison to wild type. Viral stocks were concentrated to optimise the number of sites of nascent viral RNA synthesis able to be detected by immunofluorescence imaging. Resin-embedded correlative light and electron microscopy (CLEM) was used to confirm RO formation by the recombinant virus. Cryo-sample preparation by plunge freezing has been used to make grids for cryo-correlative light and soft X-ray tomography (SXT, B24, Diamond Light Source), correlative cryo-focused ion beam (FIB) milling, and cryo-electron tomography (ET, eBIC, Diamond Light Source).
Results
Recombinant GFP-nsp2-IBV does not significantly attenuate viral replication. GFP-nsp2 colocalises with sites of nascent viral RNA synthesis as seen by immunofluorescence imaging. GFP-nsp2 marks known IBV RO structures as seen by CLEM. Correlative cryo-SXT has revealed RO networks in whole cells to 30nm resolution. Plus, correlative fluorescence microscopy, FIB milling, and cryo-ET has resolved the structure of the DMS bilayer. Sub-volume averaging on cryo-electron tomograms will gives clues to the DMS protein composition and their functions.
Conclusion
Fluorescent-tagged viruses are being used in combination with advanced imaging to further understand CoV ROs. This work will significantly improve our knowledge about the sites of CoV RNA synthesis and this critical stage of the virus lifecycle. Consequently, novel replication mechanisms of current and emerging positive-sense single-strand RNA viruses may be revealed.



Multi-scale correlative cryo-bioimaging of coronavirus replication organelles.
1-2: Replication organelle (RO) network of double membrane vesicles (DMVs) and double membrane spherules (DMSs) observed throughout an entire Infectious Bronchitis Virus-infected cell by correlative cryo-soft X-ray tomography. 3-7: ROs and associated proteins observed by cryo-electron tomography using a correlative workflow.

Keywords:

Correlative imaging, cryo-electron tomography, coronavirus

Reference:

Maier, H. J., Hawes, P. C., Cottam, E. M., Mantell, J., Verkade, P., Monaghan, P., Wileman, T., & Britton, P. (2013). Infectious bronchitis virus generates spherules from zippered endoplasmic reticulum membranes. mBio, 4(5), e00801–e813. https://doi.org/10.1128/mBio.00801-13

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Structure of the first isolated polinton-like virus and its host

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Poster Group 2

Viruses in the PRD1/Adenovirus (AdV) -like lineage infect bacteria, archaea and eukaryotic organisms and build their capsids with proteins with beta-jelly rolls orthogonal to the capsid surface (1). It has been suggested that Polintons (dsDNA transposons) are members of this lineage, because they encode genes very similar to viral proteins involved in replication, assembly, maturation, and possible structural proteins with jelly roll fold. These observations prompted the hypothesis that Polintons may have evolved from a PRD1-like ancestor (2). Subsequent evolution would have resulted in the "polintovirus" elements splitting into two different ways of life: the transposable, capsid-less integrating elements, and the bona fide viruses. Marine metagenome analyses have revealed a group of putative polinton-like viruses (PLVs) in eukaryotes. PLV genomes contain genes for single and double jelly roll proteins and a packaging ATPase, but lack the protease and integrase genes present in the polintons (3). Therefore, PLVs could represent a minimal version of the PRD1/AdV-like lineage in eukaryotic hosts. The study of this kind of virus could reveal the adaptations necessary for the jump from a bacterial to eukaryotic host. The first isolated PLV, TsV-N1, infects the unicellular eukaryotic algae Tetraselmis striata (4)

We have used cryo-electron microscopy to solve the structure of TsV-N1, and FIB-SEM volume electron microscopy on resin-embedded cells to analyse the structural changes induced by infection in the host. The high-resolution capsid structure (2.7 Å) corroborates the placement of TsV-N1 in the PRD1/AdV-like lineage and reveals similarities with those of bacteriophages in this lineage, although TsV-N1 infects a eukaryotic host. These similarities include: triangulation number T = 21d, size, genome arranged in concentric rings. However, the main differences with bacteriophages in this lineage are the absence of an inner membrane, the major capsid protein fold being more similar to other eukaryotic viruses, a complex cementing protein network and a high packing fraction, more similar to those of HK97-like viruses. TsV-N1 induces large changes in the host during the infection. Although this virus assembles in the nucleus, other cellular structures are modified too. Particularly striking is the disappearance of starch grains and the cell wall, suggesting that the virus hijacks energy sources for propagation. More studies must be carried out to understand how this virus reaches the nucleus. However, its similarities with AdV (no membrane, nuclear assembly) suggest that similar genome delivery mechanisms may be involved.

Keywords:

Polinton-like virus, cryo-EM, cellular-sections, volume-EM.

Reference:

[1] Krupovic et al., Nat Rev Microbiol 6, 941 (2008).
[2] Krupovic et al., Nat Rev Microbiol 13, 105 (2015).
[3] Yutin et al., BMC Biol 13, 95 (2015).
[4] Pagarete et al., Viruses 7, 3937 (2015).

[5]Supported by grants PID2019-104098GB-I00 and PID2022-136456NB-I00/AEI/10.13039/501100011033, CRIOMECORR (ESFRI-2019-01-CSIC-16), and BAG bi22006 at eBIC center.

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Visualizing microbial interactions and CRISPR-Cas interference using FISH applied to environmental archaeal biofilms

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Poster Group 2

Background incl. aims
Identifying microbial virus-host interactions is essential to understand viral dynamics in ecosystems and their impact on nutrient cycling. In environmental samples, their identification relies mostly on bioinformatic approaches, i.e., detecting matches between spacers encoded in clustered regularly interspaced short palindromic repeats (CRISPR) systems and their corresponding protospacers present in viral genomes. To complement and confirm these predictions, we established sophisticated microscopy techniques. First, by employing virus-targeted direct-gene fluorescence in situ hybridization (virusFISH), we demonstrated the lytic life cycle of an in silico predicted virus to infect Candidatus Altiarchaeum hamiconexum, an uncultivated free-living, biofilm-forming archaeon, via fluorescence microscopy. Secondly, we characterized the morphological changes of altiarchaeal virocells, i.e. virus-infected prokaryotic cells, using correlative fluorescence and scanning electron microscopy. This effort culminated in a protocol to link a viral genome to the ultrastructure of its virus-like particle and to co-localize these with their host. However, the CRISPR-Cas immune response in single environmental virocells remains unexplored due to current technical constraints.
Methods
As a next step in expanding our microscopy toolkit for the study of virus-host interactions in environmental samples, we utilize various FISH-based methods to visualize the presence and activity of CRISPR-Cas systems within a biofilm of Ca. Altiarchaeum hamiconexum. In the first step, we developed HRP-probes to target the host crRNA of 11 abundant spacer that target a host-specific lytic virus. As the CRISPR spacer diversity in altiarchaeal biofilm is complex, the CARD-FISH based amplification method enhances the signal intensity of low-abundant crRNA in environmental samples.
Furthermore, we developed a distinct set of probes to traget the mRNA of the cas3 protein. Amplification of the signal was achieved by employing 50 different oligonucleotide probes, each fluorescently labeled with so called FLAP tails, targeting the same mRNA segment.
The established protocol can also be combined with virusFISH, allowing a nuanced perspective on the dynamic interplay between the CRISPR system and actively infecting viruses in a natural ecosystem.
Results
Our findings uncover the spatial expression of specific CRISPR spacer sets targeting one virus population within an environmental archaeal biofilm, with a confluence of the most abundant spacers targeting the same virus covering nearly the entire biofilm. Additionally, we are able to detect mRNA of the Cas3 protein within our environmental sample. Our two independent fluorescence microscopy methods enabled us detecting active CRISPR-Cas systems in environmental samples along with the targeted viral genomes.
Conclusions
Spacers targeting the same virus within a biofilm can be heterogeneously expressed across the individual cells, resulting in an active defense of the population. Nevertheless, active infections are observed in these biofilms, suggesting an ongoing competition between the host defense system and

the virus. In sum, our results and newly developed techniques open novel avenues to understand the intricate relationships between viruses and their microbial hosts.

Keywords:

Microbiology, virology, FlSH, CRISPR, archaea

Reference:

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2. Rahlff, J. et al. Lytic archaeal viruses infect abundant primary producers in Earth's crust. Nat Commun 12, 4642 (2021).
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Interaction of transduction enhancing peptide nanofibrils with cells and virions assessed by complementary EM techniques

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Poster Group 2

Background and aims:
Retroviral gene therapies are applied to treat cancers. In chimeric antigen receptor (CAR) T-cell therapy the gene for the fusion protein CAR is inserted into the patients' T-cell genome by retroviral transduction in vitro. After reintroduction into the patient, the reprogrammed T-cells expressing CAR are able to recognize and destroy cancer cells (Cappell and Kochenderfer, 2023). For cost efficient application of CAR T-cell therapy, researchers aim at enhancing transduction efficiencies. Transduction efficiencies are often low due to the low retroviral titers. Another reason is the electrostatic repulsion between the negatively charged virions and the negatively charged plasma membrane. To overcome this repulsion, positively charged peptide nanofibrils (PNF) were developed. One type of PNF, enhancing factor C (EF-C), is actively engaged by cellular protrusions and leads to a 9.3-fold enhanced transduction rate in HeLa cells (Schütz et al., 2021). However, EF-C fibrils formed large aggregates, which could clog the bloodstream when introduced into the patient and are, thus, not suitable for clinical application. A novel peptide, Derivative-4 (D4), was identified by in-silico screening (Rauch-Wirth et al., 2023). To better understand the morphology of D4 PNF and to characterize their interaction with virions and cells, electron microscopy was performed.
Methods:
The morphology of D4 PNF was characterized by negative staining and transmission electron microscopy (TEM). The binding of D4 to virions and the cell surface was then studied by scanning electron microscopy (SEM) of cells after critical point drying. To obtain a more complete view of binding of D4 to cells, samples were analyzed by TEM and STEM tomography. For both, high-pressure frozen and freeze-substituted cells were used (Bergner et al., 2022).
Results:
TEM showed that D4 PNF are shorter than EF-C. In cell culture, most of the D4 aggregates were formed by structures measuring up to a few hundred nanometers in length ("D4 network"). SEM analysis revealed that D4 networks bind to cells via cellular protrusions. When the networks were considerably smaller than the cell, the plasma membrane formed indentations close to the network, indicated starting cellular uptake. TEM analysis of cell cultures treated with PNF and virions revealed a second D4 morphology; besides D4 networks also aggregates of µm-long, thick D4 nanofibrils were found. We then analyzed virion binding patterns to these different D4 morphologies by TEM and STEM tomography. Irrespective of the D4 morphology, virions bound to D4 aggregates. However, the D4 morphology appeared to influence virion binding. Virions bound to the surface of the D4 networks and were also found within the network. In contrast, at aggregates formed by the long and thick D4 fibrils, virions were only found on the surface of the aggregate.
Conclusion:
Using 4 complementary EM techniques, we obtained a better understanding of the transduction enhancing function of D4. This understanding is important for the further development of D4 PNF and their clinical applications.

Keywords:

STEM tomography, SEM, gene therapy

Reference:

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Schütz et al., 2021, Viral Transduction Enhancing Effect of EF-C Peptide Nanofibrils Is Mediated by Cellular Protrusions. Advanced Functional Materials. doi: 10.3389/fimmu.2023.1270243.

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Structural basis of rotavirus spike proteolytic activation

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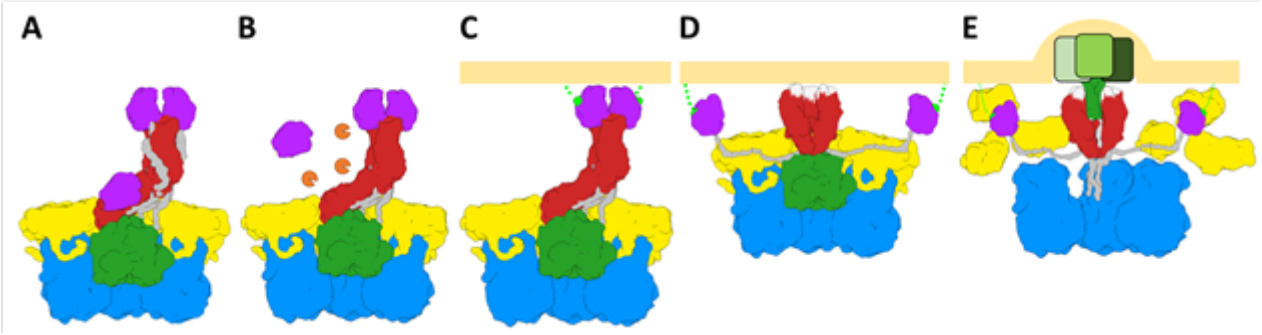
Poster Group 2

Rotavirus (RV) is the leading cause of severe gastroenteritis with dehydration in children under 5 years of age and causes ~590 million infections per year in all age groups. Rotavirus infectivity depends on the activation of viral particles by spike proteolysis by trypsin-like proteases. Although it has been described how the entry into the cell is mediated by a conformational change in the spike, the molecular mechanisms underlying this proteolytic activation process are unknown. Using cryoEM we have resolved the structure of the infective particle of group A RV before and after proteolytic activation. Despite the low occupancy and high flexibility of the spike, we have built an atomic model of both spikes by combining various computational methods. The resolved structures show that the conformation of the non-proteolyzed spike is conditioned and limited by the position of the loops that surround its structure and that join the lectin domains that form the head of the spike with its body. The proteolysis of these loops breaks this structural limitation allowing the transformation of the spike to a state competent to make the necessary conformational changes to penetrate the cell membrane.



Keywords:

Rotavirus, cryo-EM, dsRNA-virus, viral entry

Reference:

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Settembre, et al. (2011). Atomic model of an infectious rotavirus particle. EMBO Journal, 30(2), 408–416.
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Shah, P. N. M. et al. (2023). Characterization of the rotavirus assembly pathway in situ using cryoelectron tomography. 31(4), 604–615.

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Elimination of HCV replication machinery early after antiviral treatment with DAA monitored by multimodal microscopy

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Poster Group 2

Background incl. aims
Hepatitis C virus (HCV) infection in cell culture constitutes an excellent model of persistent infection whereby the virus takes control of the infected cell without killing it. This strong interference with host cell homeostasis is manifested by a profound remodeling of the host endomembrane system as well as with a strong induction of virtually all stress response pathways in the cells. The availability of specific direct-acting antiviral (DAA) drugs against HCV provides a unique opportunity to revert this process and to define the ultrastructural events that follow viral replication blockade short after antiviral treatment.
Methods
Using confocal immunofluorescence and transmission electron microscopy (TEM) as well as the correlation of cryo-fluorescence microscopy and cryo-soft X-ray tomography (cryo-FM-SXT), we monitored the HCV replication machinery removal after antiviral treatment with DAA of a surrogate cell culture model of viral replication.
Results
To assess the impact of antiviral treatment of HCV-replicating cells, we treated cells bearing an HCV subgenomic replicon with a DAA combination of sofosbuvir, a polymerase inhibitor targeting NS5B, and daclatasvir, an NS5A-targeting antiviral. Analysis of DAA-treated HCV replicons indicate that most viral antigens and RNA are eliminated within the first 48 hours of treatment, concomitant with the reversion to baseline expression of HCV-induced stress markers, such as ATF3. A general survey of control cells and HCV replicons using correlative cryo-FM-SXT indicates that HCV-induced membranous alterations are no longer visible after 24 hours of treatment and that a substantial fraction of NS5A, a viral component of the replicase is located in pleomorphic, high-absorption contrast organelles in DAA-treated cells. Three-dimensional reconstruction of these cells suggest that these organelles are spatially organized in layers proximal to the cell nuclei in areas with reduced mitochondrial content. TEM and cryo-FM-SXT studies confirmed the rapid elimination of the viral machinery, and the concurrent appearance of large endo-lysosomes and multivesicular bodies, suggesting a major role for this recycling machinery in the elimination of HCV-induced membranous compartments. These and results by others suggest that HCV replication compartment is constantly recycled by the endo-lysosomal system and that this equilibrium is unbalanced by DAA treatment, resulting in a transient activation of the endo-lysosomal system to achieve rapid viral machinery removal.
Conclusions
Overall, these TEM and correlative cryo-FM-SXT studies suggest that HCV replication machinery removal after DAA treatment entails transient proliferation of endo-lysosomes and MVB, but not that of double-membrane autophagosomes. Moreover, live fluorescence confocal microscopy indicates that NS5A remnants co-localize with an acidic compartment labeled with lysotracker green. Given that a fraction of NS5A is found in endo-lysosomes also before antiviral treatment it is reasonable to propose that HCV replicase compartment size is balanced by a constant flux through lysosomal/MVB compartments.

Keywords:

HCV, DAA, cryo-FM-SXT, TEM, recycling-machinery

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Synthetic Bispecific RBD Antibody Effectively Neutralizes SRAS-CoV-2

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Poster Group 2

Background incl. aims
Coronavirus Disease 2019 (COVID-19) pandemic is severely impacting the world, and tremendous efforts have been made to deal with it. Despite many advances in vaccines and therapeutics, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants remains an intractable challenge. We employed non-antibody protein binder called repebody and designed a dimeric form of repebody A6 which can effectively neutralizes viral infection.
Methods
Using a library of non-antibody protein binder called repebody, and rational maturation, we have produced an A6 repebody that binds RBD (KD of 2.2nM) and blocks its interaction with ACE2. By using both cryo-EM and X-ray crystallography, we successfully determined A6 repebody mode of action. We utilized ITC, SPR to measure the binding kinetics and FRNT to test A6 neutralizing ability. In-vivo neutralization test was conducted on the mouse model expressing human-ACE2.
Results
We present a bivalent Receptor Binding Domain (RBD)-specific synthetic antibody, specific for the RBD of wild-type (lineage A), developed from a non-antibody protein scaffold composed of LRR (Leucine-rich repeat) modules through phage display. We further reinforced the unique feature of the synthetic antibody by constructing a tandem dimeric form. The resulting bivalent form showed a broader neutralizing activity against the variants. The in vivo neutralizing efficacy of the bivalent synthetic antibody was confirmed using a human ACE2-expressing mouse model that significantly alleviated viral titer and lung infection.
Conclusion
In the present study, we have demonstrated that a bivalent repebody, specific for the SARS-CoV-2 RBD, effectively neutralizes the SARS-CoV-2 virus and its variants. Despite their small size, target-specific synthetic antibodies developed from the repebody scaffold through phage display offer larger interface areas. The present approach can be used to develop a synthetic antibody showing a broader neutralizing activity against a multitude of SARS-CoV-2 variants.

Keywords:

SARS-CoV-2, Repebody, Cryo-EM, Synthetic antibody

Reference:

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Investigating VSV-GP replication using TEM & STEM tomography of high pressure frozen, freeze substituted samples

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Poster Group 1

Background: With a growing demand of viral products for gene therapy and as oncolytic agents, understanding the production process of viruses in the biopharmaceutical environment becomes increasingly important. For this, besides controlling classical bioprocess parameters like infectious titer, there is a need for additional methods to characterize the Drug product. Up to now, electron microscopy (EM) remains the only way of directly visualizing cellular processes during virus replication. Here, we aimed at the development of a protocol using high pressure freezing and subsequent freeze substitution for studying manufacturing of VSV-GP, a recombinant vesicular stomatitis virus (VSV) pseudotyped with the glycoprotein of lymphocytic choriomeningitis virus (LCMV-GP).
Methods: Transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) tomography were used to characterize VSV-GP replication kinetics. Samples were prepared by high pressure freezing and subsequent freeze substitution. The ultrastructure was compared with the number of viral genomic copies, quantified by qPCR.
Results: High pressure freezing and freeze substitution of chemically fixed VSV-GP infected cells yielded a good ultrastructural preservation. At 8 hours post infection, inclusion bodies and bullet shaped virions budding from the plasma membrane could be observed. TEM and STEM tomograms indicate a colocalization of inclusion bodies and rough endoplasmic reticulum.
Conclusion: A hybrid fixation by chemical inactivation and subsequent high pressure freezing proved feasible for VSV-GP infected cells. Three-dimensional reconstruction of STEM tomograms yields information on VSV-GP replication that is otherwise not accessible by TEM imaging and that improves the scientific understanding of viral genome replication and virion morphogenesis.

Keywords:

High pressure freezing, freeze substitution

Reference:

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