with virus uptake and intracellular fusion kinetics. This allows us to draw a comprehensive picture of the first steps of virus infection. Further we can draw conclusions about the infectivity and could explain why some cells are permissive while others are not.

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Elucidating the Mechanism of Ebola Virus Assembly and Budding

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Ebola virus from the filoviridae family is characterized by internal and external bleeding in primates due to coagulation abnormalities induced by the virus at the onset of the infection. With no vaccines or treatment, Ebola is classified as bio-safety level IV agent with the potential to be used as a biological weapon. Details of virus assembly are poorly understood. Evidence suggests that matrix protein VP40 is the main driving force for assembly and budding. Generation of new virus involves a cascade of cellular events that recruit the viral genome, the matrix proteins and subsequent acquisition of the viral envelope from the host cell. The new virus like particle (VLP) forms at a bud site at the inner leaflet of the plasma membrane and can serve as a primary therapeutic target for inhibiting Ebola virus replication. Preliminary results demonstrate that VP40 alone assembles in mammalian cells into VLPs independent of other viral proteins. It binds to the plasma membrane with nanomolar affinity and possesses the ability to modify membrane structure. VP40 induces membrane curvature changes, an important step for bud formation and egress of the newly formed virus. This project is aimed at elucidating the mechanistic details of VP40 assembly on the plasma membrane using an interdisciplinary approach. Specifically, we have employed in vitro lipid binding and curvature assays with cellular scanning and single molecule microscopy to investigate the basis of VP40 lipid binding, membrane bending and viral egress. Our results further demonstrate that VP40 oligomerizes on the plasma membrane in a PS-dependent manner and also remodels actin network for assembly and maturation. Our results represent a key step to understanding the general principles governing the remodeling of membrane by matrix proteins from lipid enveloped viruses such as Ebola and HIV.

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Revealing the Impact of Fluorescent Labeling on HIV-Gag Virus-Like Particle Formation by Quantitative Super-Resolution Imaging and Fluorescence Correlation Spectroscopy

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A viral infection is ultimately marked by the assembly and release of progeny viral particles. In the case of HIV-1 this process is driven by Gag. When expressed in cells Gag is sufficient to assemble virus-like particles (VLPs) of radius ~70 nm. During this process, the number of Gag proteins increases over several orders of magnitude, from a few at nucleation to thousands at completion. The assembly process has been studied using fluorescently labeled Gag, but expressing labeled Gag alone can lead to aberrant VLP morphology. It has been proposed that this aberration can be rescued by an additional expression of unlabeled Gag. However, the impact of unlabeled to labeled Gag stoichiometry on the morphology of VLPs has not been quantified. This is due to two main limitations: the lack in quantification of the Gag protein stoichiometry in single cells, and the range of sizes of forming VLPs. Indeed, methods such as standard fluorescence or electron microscopy cannot access all stages of the VLP assembly process. To address these limitations we first quantify the labeled/unlabeled Gag stoichiometry in single cells using fluorescence correlation spectroscopy and a reporter protein for unlabeled Gag. We then demonstrate an approach using super-resolution imaging (SR) that permits quantitative morphological and molecular counting analysis. We use this approach on mammalian cells expressing Gag labeled with mEos2, the tandem dimeric tdEos or a mixture of labeled and unlabeled Gag. Gag cluster shapes are analyzed and the number of Gag proteins per cluster is extracted. We show that for mEos2 the label has no impact on VLP morphology, whereas tdEos leads to an increase in VLP size, which can be rescued by additional expression of unlabeled Gag.

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Temporal and Spatial Remodeling of Host Cell Plasma Membrane during HIV Assembly Revealed by Quantitative Superresolution Microscopy

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The budding of enveloped retrovirus, such as HIV, involves oligomerization of the structural protein Gag at the plasma membrane and the incorporation of a patch of the host cell plasma membrane in the viral particle. Previous studies indicate that certain host proteins and viral envelope proteins are enriched in the membrane of virus particles. Since the viral membrane is derived from the host membrane, such specialized composition of viral membrane suggests viral assembly and budding involve differentiation of the host cell plasma-membrane at the assembly site. However, the mechanism of the membrane remodeling process during viral assembly is not understood. using a combination of quantitative superresolution microscopy (PALM and STORM) and diffraction-limited TIRF microscopy, we have investigated the spatial and temporal differentiation of the host cell plasma-membrane during budding of HIV. We find that in absence of Gag, the viral envelope protein is randomly distributed in the plasma membrane. However, oligomerization of Gag at the membrane leads to dramatic redistribution of the envelope proteins at the site of Gag assembly. We also see enrichment of GPI-anchored protein such as CD59 at the assembly site, while a model transmembrane protein EGFP-GT46 is actively excluded from the membrane patch. Gag is anchored to the plasma membrane via myristoylated lipid anchor and electrostatic interactions. Interestingly, we find that myristoylated inner leaflet anchored proteins are enriched while proteins with farnesyl and geranyl lipid anchors are depleted at the assembly site. Taken together, our results indicate that the oligomerization of HIV Gag at the plasma-membrane creates a specialized microenvironment, which leads to the differentiation of the local plasma membrane. This provides new insights about the temporal and spatial remodeling of plasma membrane during assembly and budding of retroviruses.

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Steps within the Assembly of HIV Gag Virus Like Particles

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HIV gag assembles at the plasma membrane and results of budding of HIV particles. We have monitored assembly of fluorescently labeled Gag molecules at the bottom surface of cells using TIRF microscopy with variable penetration depths. In this work we present quantitative analysis of intensity of vesicle like particles (VLPs) which form with fluorescently-tagged Gag on the cell membrane. To verify that these vesicles actually represent fully coated VLPs that separate from the host cell we have monitored the recruitment of VPS4-mCherry which is a critical member of the Endosomal Sorting Complexes Required for Transport (ESCRT) and its recruitment indicates fission from the host cell. Since the polymerization of Gag is a constant process, we expect the fluorescence intensities to increase continuously over time until it reaches a maximum, which signifies that the VLP is complete. However, we have found that the intensities of these VLPs increase in discrete steps. In order to further investigate the frequency and distribution of these pauses we have developed a mathematical model of Gag polymerization during virus assembly. This model is based on the spherical geometry of a VLP and how the total number of Gag proteins changes as the sphere forms. We have used least squares regression to determine the best fit of the model to the data, which we have then used to consider how these pauses have affected the total assembly time of the VLPs.

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Curvature Stimulates Assembly of Gag Shell through Distinct Fluid-Like Intermediate

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HIV particles assemble on the plasma membrane of a host cell. While the plasma membrane provides an integrative platform for the virus assembly, it also constitutes a mechanical barrier for viral budding and fission. Gag, the polyprotein forming a shell lining the envelope membrane of HIV, is sufficient to overcome this barrier: self-assembly of Gag into small clusters on the membrane surface leads to membrane bending and, ultimately, to production of virus-like membrane particles. The mechanisms behind membrane curvature