Quantification and a Molecular Dynamics Study of Viral Membrane Lipids through Plasmon Coupling Microscopy

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ABSTRACT

Phosphatidylserine (PS) and monosialotetrahexosylganglioside (GM1) are examples of two host-derived lipids in the membrane of enveloped virus particles that are known to contribute to virus attachment, uptake, and ultimately dissemination. A quantitative characterization of their contribution to the functionality of the virus requires information about their relative concentrations in the viral membrane. Here, a gold nanoparticle (NP) binding assay for probing relative PS and GM1 lipid concentrations in the outer leaflet of different HIV-1 and Ebola virus-like particles (VLPs) using sample sizes of less than 3×10⁶ particles is introduced. The assay evaluates both scattering intensity and resonance wavelength and determines relative NP densities through plasmon coupling as a measure for the target lipid concentrations in the NP-labeled VLP membrane. In addition, the mechanical properties of the viral membrane have been found to be contributing to the efficient reproduction cycle of the virus. Membrane fluidity which is a function of temperature and membrane composition is one of the crucial factors in viral activity. We have used temporally-resolved microscopy on silver NPs to track these molecular dynamics.

METHODS and RESULTS

INTRODUCTION

• Enveloped viruses are a class of viruses that are enveloped in a lipid bilayer.
• It is becoming increasingly clear that the host-derived membrane of an enveloped virus contributes more to the infection mechanism than simply forming a molecular scaffold for the presentation of virus encoded membrane glycoproteins.
• Phosphatidylserine (PS), for instance, has been shown to facilitate apoptotic mimicry and enhance glycoprotein-independent uptake of Vaccinia, Ebola and Dengue viruses.
• Glycosphingolipids (GSLs) are another important class of lipids that mediate interactions between virus particles and host cells.
• Lipids contribute significantly to mediating virus – host-cell interactions, and this realization has motivated great interest in a quantitative analysis of viral lipidome to identify potential diagnostic and therapeutic targets.

The structure of an enveloped virus particle

NP labeling strategy for PS and GM1. a) PS or GM1 are labeled using AnxV or CTB in combination with Biotin-Neutravidin binding chemistries. b) TEM images of fixed and negatively-stained VLPs after NP binding to PS. Scale bars are 40 nm. c) 29/31KE VLPs by mDCs.

Mutations in the matrix domain of the Gag redirect the formation of VLPs from plasma membrane to intracellular compartments. Fluorescence images of HEK293T cells 1 day post transfection with a) WT Gag-eGFP, b) ΔMA Gag-eGFP and c) 29/31KE Gag-eGFP expression plasmids. Plasma membrane (GM1) and nucleus were stained in red and blue, respectively. d) Relative capture of WT, ΔMA and 29/31KE VLPs by mDCs.

Optical set-up for correlated fluorescence / multispectral darkfield imaging. a) Scheme of the optical set-up to characterize NP labeled VLPs (inset), 1-Tungsten lamp, 2-Filter wheel, 3-Darkfield condenser, 4-25x objective, 5-60x oil objective, 6-Fluorescence filter set, 7-EMCCD.

Relative Labeling efficiencies for different lipid-VLP combinations: PS-WT, PS-GM1-WT and control.


Optical quantification of PS and GM1 contents in 4 different HIV-1 VLPs. 

- Optical quantification of PS and GM1 contents in 4 different HIV-1 VLPs.
  - I_scatter versus λ_res scatter plot after labeling PS (top row) and GM1 (bottom row) in (from left to right) WT, PDMP, ΔMA and 29/31KE VLPs. Each plot contains the data of 500-1500 VLPs obtained in 3 or more independent experiments with one batch of VLPs. Data are plotted as black markers and fitted distribution functions, $P(I_{\text{scatter}}, \lambda_{\text{res}})$, are overlaid as color maps.
  - $F$ values for lipid-VLP combinations shown in (a) and a WT VLP negative control that lacked any AnxV or CTB treatment.
  - PS and GM1 concentrations (in mol%) resulting from the optical measurements.

In conclusion, we have introduced a new optical assay for measuring the lipid contents in VLP and viral envelope membranes based on the spectral analysis of gold nano-labelling and plasmon coupling. In addition, based on the same lipid targeting strategy, we are able to track translational and rotational lipid motions in the membrane of virus particles with time-resolved scattering spectroscopy and scattering polarization fluctuation microscopy of silver NP labels. We believe these techniques can contribute significantly to understanding the underlying mechanisms of virus-cell interactions.

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