

Abstract

# Specific Lipid Recruitment by the Retroviral Gag Protein upon HIV-1 Assembly: From Model Membranes to Infected Cells <sup>†</sup>

Cyril Favard <sup>1</sup>, Jakub Chojnacki <sup>2</sup>, Naresh Yandrapalli <sup>3</sup>, Johnson Mak <sup>4</sup>, Christian Eggeling <sup>5,6</sup> and Delphine Muriaux <sup>1,\*</sup>

<sup>1</sup> Montpellier Infectious Disease Research Institute (IRIM), CNRS—University of Montpellier, 1919, Route de Mende, 34293 Montpellier CEDEX, France; cyril.favard@irim.cnrs.fr

<sup>2</sup> IrsiCaixa AIDS Research Institute, University Hospital Germans Trias i Pujol, Ctra. de Canyet s/n, Badalona, 08916 Barcelona, Spain; jchojnacki@irsicaixa.es

<sup>3</sup> Team Biomicrofluidic Systems, Max Planck Institute of Colloids and Interfaces, Potsdam-Golm, Germany; Naresh.Yandrapalli@mpikg.mpg.de

<sup>4</sup> Institute for Glycomics, Griffith University Gold Coast, Southport, QLD 4215, Australia; j.mak@griffith.edu.au

<sup>5</sup> Leibniz Institute of Photonic Technology e.V., Albert-Einstein-Straße 9, 07745 Jena, Germany; christian.eggeling@uni-jena.de

<sup>6</sup> Institute of Applied Optics and Biophysics, Friedrich-Schiller-University Jena, Max-Wien Platz 4, 07743 Jena, Germany

\* Correspondence: delphine.muriaux@irim.cnrs.fr

<sup>†</sup> Presented at Viruses 2020—Novel Concepts in Virology, Barcelona, Spain, 5–7 February 2020.

Published: 24 June 2020

**Abstract:** The retroviral Gag protein targets the plasma membrane of infected cells for viral particle formation and release. The matrix domain (MA) of Gag is myristoylated for membrane anchoring but also contains a highly basic region that recognizes acidic phospholipids. Gag targets lipid molecules at the inner leaflet of the plasma membrane including phosphatidylinositol (4,5) bisphosphate (PI(4,5)P<sub>2</sub>) and cholesterol. Here, we addressed the question whether HIV-1 Gag was able to trap PI(4,5)P<sub>2</sub> and/or other lipids during HIV-1 assembly *in silico*, *in vitro* on reconstituted membranes and *in cellulo* at the plasma membrane of the host CD4<sup>+</sup> T cells. *In silico*, we could observe the first PI(4,5)P<sub>2</sub> preferential recruitment by HIV-1 MA or Gag while protein docked on artificial membranes. *In vitro*, using biophysical techniques, we observed the specific trapping of PI(4,5)P<sub>2</sub>, and, to a lesser extent, cholesterol and the exclusion of sphingomyelin, during HIV-1 myr(-)Gag self-assembly on LUVs and SLBs. Finally, in infected living CD4<sup>+</sup> T cells, we measured lipid dynamics within and away from HIV-1 assembly sites using super-resolution stimulated emission depletion (STED) microscopy coupled with scanning Fluorescence Correlation Spectroscopy (sSTED-FCS). The analysis of HIV-1 infected CD4<sup>+</sup> T lymphocytes revealed that, upon virus assembly, HIV-1 is able to specifically trap PI(4,5)P<sub>2</sub>, and cholesterol but not phosphatidylethanolamine (PE) or sphingomyelin (SM) at the cellular membrane. Furthermore, analyzing CD4<sup>+</sup> T cells expressing only HIV-1 Gag protein showed that Gag is the main driving force restricting the mobility of PI(4,5)P<sub>2</sub> and cholesterol at the cell plasma membrane. Our data provide the first direct evidence showing that HIV-1 Gag creates its own specific lipid environment for virus assembly by selectively recruiting lipids to generate PI(4,5)P<sub>2</sub>/cholesterol-enriched nanodomains favoring virus assembly, and that HIV-1 does not assemble on pre-existing lipid domains.

**Keywords:** HIV-1 assembly; Lipid nanodomains; STED microscopy

---



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).