Measuring the membrane order of the inner leaflet in the HIV-1 Gag assembly site

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Résumé

Human immunodeficiency virus type 1 (HIV-1) is an enveloped virus that acquires host lipid during budding from the host plasma membrane (PM). Lipidomics studies have shown that virions have a different lipid composition from PM, characterized by enrichment in sphingomyelin (SM), cholesterol (Chol), phosphatidylinositol-(4,5)-bisphosphate (PIP(4,5)P2), and phosphatidylserine (PS). During the late phase of the viral replication cycle, Gag is targeted to the PM via myristoylation and positive charges within the matrix (MA) domain. Subsequently, Gag multimerizes to form a Gag lattice with membrane curvature as a platform for viral assembly. Notably, Gag expression alone is sufficient to drive the formation of virus-like particles (VLPs).
In the PM, lipids are asymmetrically distributed across the bilayer. SM is mainly found in the outer leaflet, whereas PI(4,5)P2 and PS are in the inner leaflet. Saturated lipids, such as SM, together with Chol, tend to form tightly packed (liquid-ordered, Lo) domains, also called "lipid rafts", creating lateral heterogeneity in the outer leaflet. In contrast, most inner leaflet lipids are (poly)unsaturated and form more loosely packed (liquid-disordered, Ld) membranes.

A major question in HIV-1 assembly is how inner leaflet Gag proteins enrich outer leaflet lipids, such as SM, into virions without direct contact. Our previous work showed that Gag brings SM-rich and Chol-rich domains into close proximity in a multimerization- and curvature-dependent manner(1). However, in living cells, the local physical properties (lipid order, polarity, and viscosity) around the Gag are largely unknown. Therefore, it is essential to determine the physical properties of the inner leaflet at Gag assemblies to understand the mechanism of Gag-induced lipid enrichment.
Here, we employed a combination of fluorescence lifetime imaging microscopy (FLIM) and a recently developed solvatochromic probe, NR12-Halo(2), alongside Halo-tagged Gag proteins (Gag-Halo) to quantitatively assess membrane order in the inner leaflet of Gag assemblies. We first confirmed that Halo tagging did not impair Gag expression and VLP production by Western blotting. We also confirmed that NR12-Halo labeling specifically localized Gag-Halo to the PM by confocal microscopy. FLIM measurements were then performed on HeLa cells expressing wild-type Gag (Gag WT) and the multimerization-deficient Gag-WM and Gag-NC mutants, in addition to controls that are preferentially partitioned in Lo and Ld domains. The fluorescence lifetimes of NR12-Halo conjugated proteins revealed that Gag WT significantly increased membrane order compared to Gag-WM and the Ld domain control. Next, to investigate the influence of membrane curvature on membrane order at the assembly, we performed FLIM imaging of cells expressing Gag mutants (Gag-P99A and Gag-EE),

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which are defective in curvature formation. The results showed that Gag-P99A and Gag-EE increased membrane order compared to Gag-WM, but not as much as Gag WT. Together, these findings suggest that both multimerization and membrane curvature are necessary to increase membrane order in the inner leaflet at Gag assembly. This study provides new insight into how Gag reorganizes the PM during HIV-1 assembly.

Mots-Clés: Virology, HIV, 1 assembly, Plasma membrane order, Environment sensitive dye, FLIM