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

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Résumé

HIV-1 is one of the viruses responsible for AIDS (acquired immunodeficiency syndrome) and remains a major global public health threat, with approximately 40 million people infected. HIV-1 has a lipid envelope acquired during budding from the host plasma membrane (PM). Several lipidomics studies have reported that the specific set of lipids, such as sphingomyelin (SM), cholesterol (Chol), phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2), and phosphatidylserine (PS), are enriched in virions. In the late phase of the viral replication cycle, viral Gag proteins are recruited to PI(4,5)P2 in the inner leaflet of the PM, forming multimeric assemblies with an inherent membrane curvature as a platform for virus assembly. In the PM, lipids are asymmetrically distributed between the outer and inner leaflet. SM is mainly found in the outer leaflet, whereas PI(4,5)P2 and PS are found in the inner leaflet. Saturated lipids, such as SM, together with Chol, tend to form tightly packed (liquid-ordered, Lo) domains called "lipid rafts", creating lateral heterogeneity in the outer leaflet. On the other hand, most inner leaflet lipids are (poly)unsaturated and form more loosely packed (liquid-disordered, Ld) domains.

A major question in HIV-1 assembly is how Gag proteins in the inner leaflet enrich outer leaflet lipids, such as SM, into virions without direct contact. Our recent results showed that Gag brings SM-rich and Chol-rich domains into close proximity in a multimerization- and membrane curvature-dependent manner(1). Our findings further raise the question of how Gag proteins communicate with outer leaflet lipids to enrich them. In vitro experiments have suggested that Gag prefers Ld domains for membrane binding. The Lo domain in one leaflet has also been shown to induce the formation of the Lo domain in the other leaflet, which is composed of Ld lipids. However, the 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 in Gag assemblies to address the issue of the lipid enrichment by Gag.

In this study, we tried to quantitatively measure membrane order in the inner leaflet of Gag assemblies using recently developed environment-sensitive dyes NR-Halo(2) and advanced microscopy.
We first established labeling conditions for the Halo-tagged Gag protein (Gag-Halo) with NR-Halo dyes in the cell. We then performed ratiometric imaging and fluorescence lifetime imaging microscopy (FLIM) of HeLa cells expressing wild-type Gag (Gag WT) and

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the multimerization-deficient Gag-WM mutant, in addition to control proteins that prefer liquid-ordered (Lo) and liquid-disordered (Ld) domains. Gag-Halo conjugated with NR12-Halo (Gag-Halo-NR12) reported that Gag WT showed a higher membrane order than Gag-WM and Ld domain control. In our previous study, Gag mutants defective in the membrane curvature formation (Gag-P99A and Gag-EE) were less effective in enrichment of lipid domains than Gag WT(1). When we measured membrane orders in the cells expressing these mutant assemblies, they showed higher membrane orders than Gag-WM and comparable to Gag WT. These results suggest that Gag multimerization increases membrane order in the inner leaflet of Gag assemblies.