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Imaging HIV-1 viral proteins by advanced fluorescence imaging techniques

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Abstract

Quantitative imaging techniques, such as FRET-FLIM, FCS and FCCS, and RICS are powerful tools to monitor the dynamics and interactions of proteins in live cells. This information is perfectly complemented by single-molecule super-resolution techniques such as PALM, STORM, and PAINT which help localizing and mapping individual molecules. We used these tools to monitor the intracellular fate of the Gag polyprotein and nucleocapsid protein (NCp7) of the Human immunodeficiency virus type 1 (HIV-1). By monitoring NCp7 during the early steps of HIV-1 infection, we evidenced that NCp7 molecules are released from viral complexes during their trafficking towards the nucleus, in synchrony with reverse transcription. We also showed that the released NCp7 molecules localize mainly in the cytoplasm and the nucleoli, where they bind to cellular RNAs. Furthermore, super-resolution PALM/dSTORM helped us defining the distribution of NCp7 molecules in the nucleoli compartments. In the late steps of HIV-1 infection, we evidenced that the Gag polyprotein interacts through its NC domain with RPL7, a major ribosomal protein involved in translation regulation. This interaction was also examined in solution, where we could show that RPL7 promoted the Gag chaperone activity, favoring the notion that Gag recruits RPL7 to overcome major roadblocks in viral assembly. Finally, we examined the dependence of the recruitement of genomic RNA by Gag and the traffic of the complex to the plasma membrane on the structure of the NC domain of Gag.

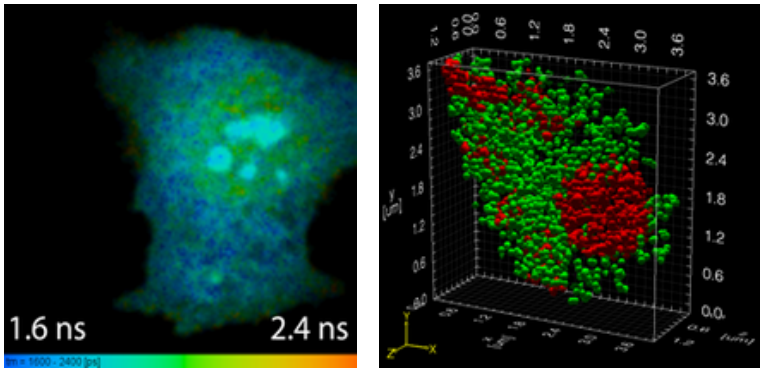

Figure : FRET-FLIM image showing the interaction of HIV-1 NCp7 with cellular nucleic acids (left); two-colour superresolution 3D image of the granular and dense fibrillar sub-domains of the nucleolus (right).