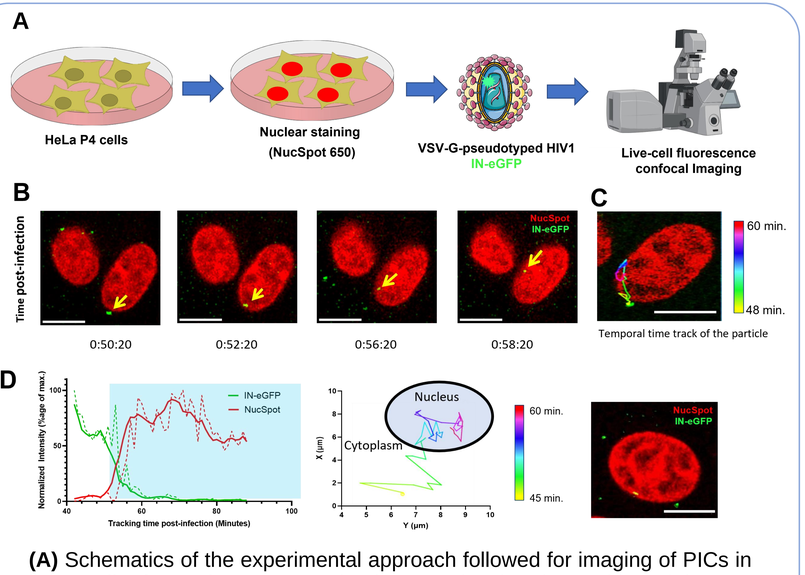
Single Virus Imaging of HIV-1 Nuclear Import

Anayat Bhat, Ivan Nombela, Barbara V. Remoortel, **Frauke Christ**, Zeger Debyser
Laboratory for Molecular Virology and Gene Therapy, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Flanders, Belgium

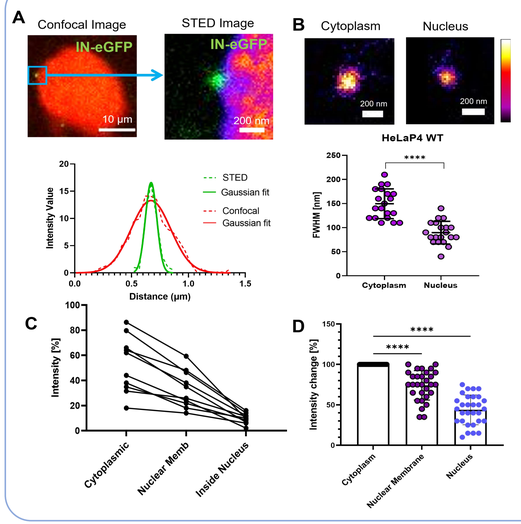
216

INTRODUCTION

The mechanism of the early steps of HIV-1 replication including capsid uncoating, nuclear import, and integration remain poorly understood. In particular, the intracellular localization and timing of capsid uncoating is a matter of debate. Conventional molecular biology techniques provide a limited understanding of the process at the ensemble level. Advanced microscopy methods offer the advantage to study the mechanism of viral replication at single virus level. We use fluorescently labelled integrase and time-lapse imaging to analyze the different steps of the replication cycle of HIV-1 in living cells. We follow the trajectory of the individual particles and their change in fluorescence intensity during their transport to the nucleus. We have also optimized super-resolution microscopy, STED (stimulated emission depletion) to estimate and compare the size of cytoplasmatic and nuclear pre-integration complexes (PICs) with a high spatial resolution. A decrease in the fluorescence intensity and size of nuclear PICs corroborates that uncoating mainly takes place at the nuclear membrane. The depletion of TRN-SR2 hampered single-round infection by 60% whereas, the decrease in single-round infection in cell lines depleted for TNPO1 or CPSF6 was not significant pointing to a possible redundancy for the use of host factors in the process of nuclear import.



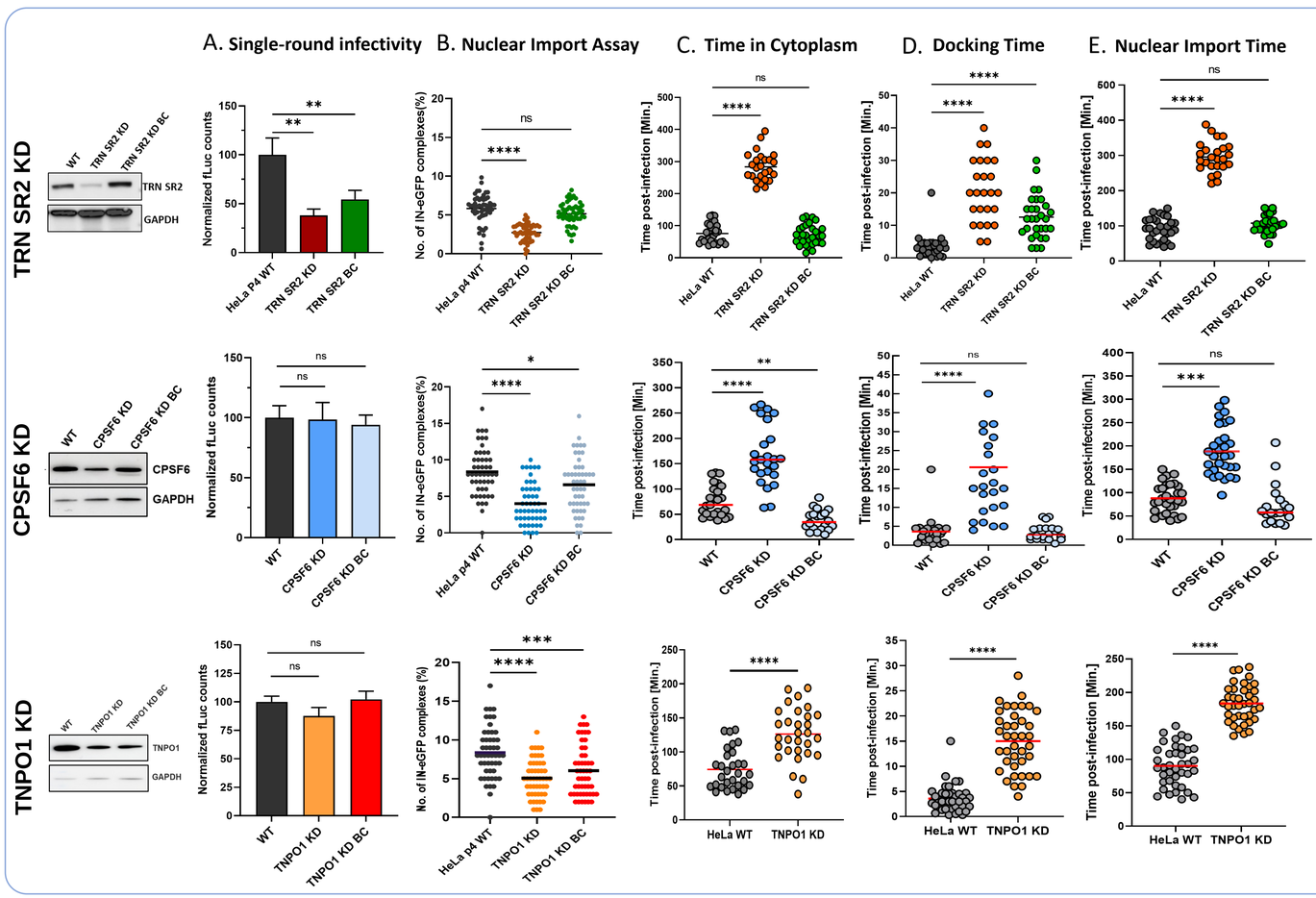
(A) Schematics of the experimental approach followed for imaging of PICs in living cells. **(B & C)** Representative images showing the position of the PIC with respect to nucleus over-time in different frames in 2D and 3D with a temporal time track. **(D)** An intensity-time plot indicating the postion of PIC w.r.t. nucleus and it's decrease in intensity over time in HeLa P4 cells. A 2D graphical representation of the particle track over time. Scale bars, 10µm.



Decrease in size and intensity of PICs upon nuclear import. (A) A representative image and intensity profile of HIV-1 particles to compare confocal and STED imaging. **(B)** STED microscopy was used to image preintegration complexex (PICs) in the cytoplasm and in the nucleus to compare their size at nanometer resolution. A decrease in the size of PICs in the nucleus is observed. **(C & D)** A decrease in fluorescence intensity of PICs is seen as they move from the cytoplasm to the nucleus by confocal microscopy. Data indicate the mean ± standard deviations. Statistical significance is determined by using an ordinary one-way ANOVA; ***P < 0.001, ****P < 0.0001.

RESULTS

- After transient docking to the nuclear membrane (1-2min) HIV PICs are imported into the nucleus.
- A 2-fold decrease in the fluorescence intensity signal and a 1.5-fold decrease in the size of nuclear PICs observed by confocal and STED imaging suggests uncoating takes place at the nuclear membrane.
- In TRN-SR2, TNPO1 and CPSF6-depleted cell lines a 2 to 3-fold increase in the docking time, the time spent in the cytoplasm, and the overall import time of PICs were observed confirming their role in nuclear import.



(A) Immunoblot to validate knock-down (KD) and back-complementation (BC) of TRN-SR2, CPSF6 and TNPO1 host-factors in HeLa P4 cells. **(B)** In a single-round infectivity assay with HIV-1 expressing firefly luciferase, the luciferase activity was measured 72 hours post-transduction. **(C)** HIV-infected cells were fixed 6 hours after infection and a decrease in the number of PICs is seen in the nuclei of depleated cells. **(C, D & E)** By single-virus imaging, PICs were tracked in living cells to measure the time spent by each PIC in the cytoplasm, the docking time on the nuclear membrane and the total nuclear import time. All these parameters increase significantly in depleted cells. The phenotype is (partially) rescued in back-complemented cells. Data indicate the mean ± standard deviations. An ordinary one-way ANOVA was used to test the statistical significance compared to HeLaP4 WT control; * P < 0.05, ** P < 0.01, ***P < 0.001, ****P < 0.0001.