Construction and Validation of a Halo-tagged fluorescence HIV-1 particles for single virus punctate imaging inside living cells

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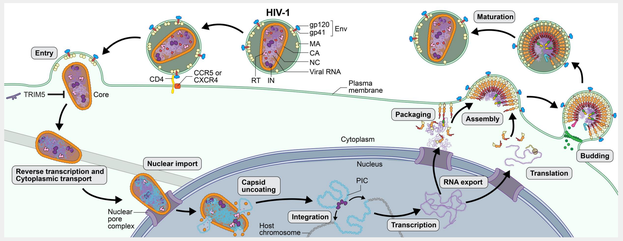
Abstract

A Halo-tag is a self-labeling protein tag used to study interactions and localization through fluorescence imaging. My UROP project was geared towards creating a Halo-tagged virus that would have the unique capabilities of fluorescing at two different wavelengths. Once the tagged virus is mixed with the cells, a microscope will enable the visualization of where the virus is and how it travels around the cell. To accomplish this goal I constructed a plasmid encoding for the Vpr-integrase-Halo tag (BruVIN-Halo) fusion protein, that when co-transfected with a viral genome, encoding plasmids can become incorporated into virions. I evaluated the incorporation of the BruVIN-Halo, and its labeling by Janelia Fluorophore (JF646) by confocal microscopy. Image analysis showed a strong labeling of the viral BruVIN-Halo protein, which appeared as a bright puncta resistant to photobleaching over ~4000 iterations of imaging. In ongoing work, I am evaluating the effects of bruVIN-Halo tag incorporation on virus infection. Subsequently, I am to infect living Hela Cells and image the virus localization and trafficking in the nucleus. These experiments will help me accurately pinpoint the localization of HIV-1 inside different compartments of the mammalian cell. I also aim to explore stochastic labeling of the IN-Halo protein, which involves colabelling the virus with 2 fluorophores, and examine molecular changes to the virus architecture by fluorescence resonance energy transfer (FRET) imaging of HIV-1 infection.



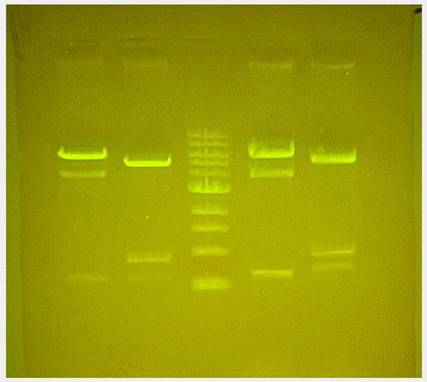
Introduction

- HIV-1 (human immunodeficiency virus) is a retrovirus that causes immunosuppression leading to AIDS (Swinkels et al. 2024)
- HIV-1 virions comprise a capsid that holds viral RNA and the reverse transcription complex of proteins necessary for integration into the host genome (Rossi et al. 2021)
- A Halo-tag is a modified haloalkane dehalogenase designed to covalently bind to synthetic ligands, which are then attached to fluorescent dyes (Marques et al. 2022)
- The interaction of the HIV-1 capsid into the host cell machinery is not completely understood, with a better understanding of these concepts can lead to treatment breakthroughs halting viral integration at crucial steps.
- We hypothesize that when the BruVIN-Halo tag is incorporated into virions, it will have the ability to fluoresce at two wavelengths

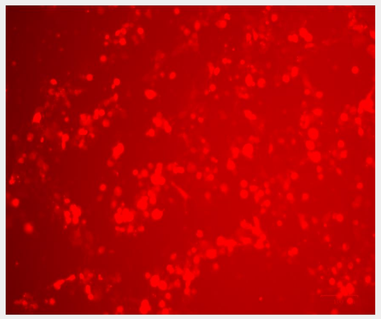
Results

Fluorescence Microscopy

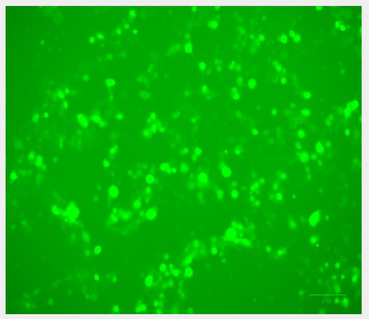
Figure 2. Fluorescent microscopy shows the ability of the Vpr-integrase-Halo tag to fluoresce at different wavelengths under the microscope, showing the integration of the HIV-1 virus inside the living cells. Yellow fluorescence shows the overlap of viral particles.



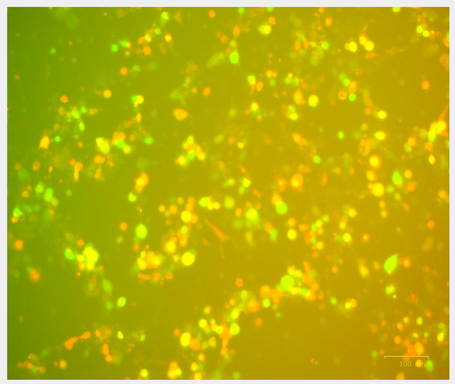
1. Digest run on agarose gel next to ladder to ensure length of sequences in the protein is accurate.



2A. Red fluorescence IN-halo-646



2B. Green fluorescence IN-halo-646



1C. Figures 1B and 1A merged, IN-halo-646

Methods

Creating the Halo Tag

- PCR from Halo template
- Digest bruVIN-mNG plasmid and retain bruVIN backbone fragment
- Ligate Halo with bruVIN backbone
- Transform JM109 competent E coli
- Isolate plasmid, digest for conformation

Incorporation of Halo-tag into virus particles

1. 1:3 diluted 293T cells plated into each well of a 6-well plate
2. Cells transfected with viral plasmids
    1. Each well received pHIVeGFP (viral backbone), BVIN (integrase), VSV-G (viral envelope), and respective BruVIN halo-tag fusion protein
3. Media of transfected cells changed 6 hours post-transfection
4. Green and red fluorescence checked under confocal microscope
5. Images were taken of the green and red fluorescence and merged together using respective programming

Discussion

- Image analysis showed a strong labeling of the viral bruVIN-Halo protein
- Appeared as a bright puncta resistant to photobleaching over around 4000 iterations of imaging
- By merging the red and green fluoresce images, we are able to see an overlap (shown in yellow fluorescence) which shows the overlap of fluoresce of the virus in the cells.
- In ongoing work I am evaluating the effects of bruVIN-Halo- tag incorporation on viral infection
- I am to infect living Hela Cells and image the virus localization and trafficking in the nucleus.
- These experiments will help me accurately pinpoint the localization of HIV-1 inside different compartments of the mammalian cell.
- I am to explore stochastic labeling of the IN-Halo protein, which involves colabelling the virus with 2 fluorophores, and examine molecular changes to the virus architecture by fluorescence resonance energy transfer (FRET) imaging of HIV-1 infection

References

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Acknowledgements

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