HIV-1 CAPSID-NUCLEAR ENVELOPE INTERACTIONS THAT FACILITATE NUCLEAR IMPORT

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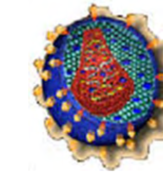

HIV Dynamics and Replication Program

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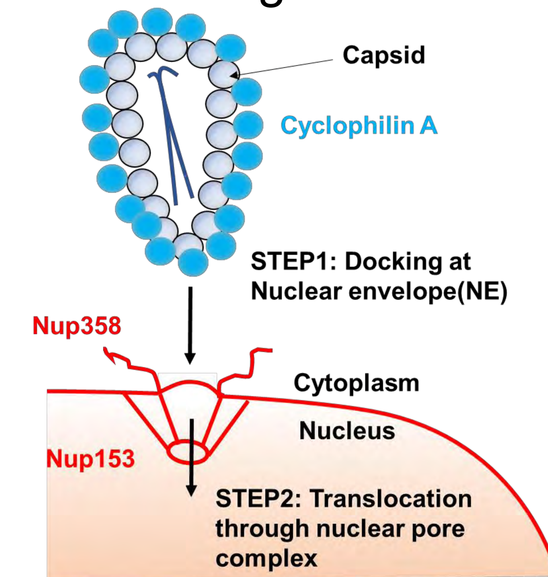
BACKGROUND

HIV-1 must enter the nucleus and integrate its DNA into host genome for successful infection. However, the mechanism by which the viral complex docks at the nuclear envelope (NE) and enters the nucleus is not well understood. Although CA is known to play a critical role in nuclear import, the CA determinants that influence NE docking and viral complex translocation through the nuclear pore have not been defined. To identify the critical CA determinants, we developed quantitative live-cell imaging assays to study the NE docking and nuclear import of single viral complexes.



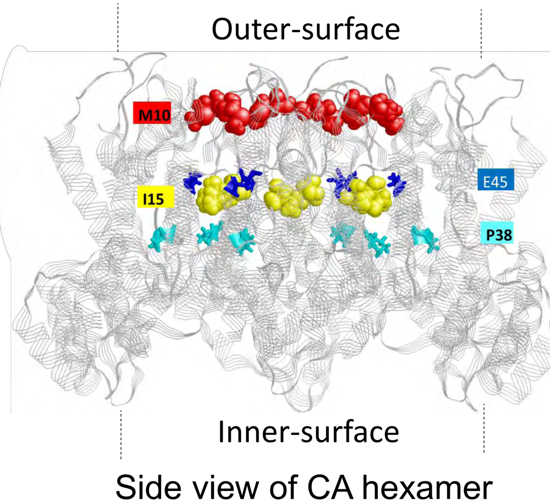
- HIV-1 replication requires import of intact HIV-1 capsid (Burdick, *et. al.* PNAS. 2020)
- The interaction of HIV-1 capsid with nuclear pore complex (NPC) can be considered as a two-step process
  1. Docking of capsid at NPC; most of the particles(~88%) that dock at NE, leave NE without being imported. Their NE residence times are <20min
  2. Translocation through NPC; a minor fraction of particles (~12%) that dock at NPC forms a stable association with it. Their NE residence times are >20min. Few of these stable associated particles manage to get imported into the nucleus

Goal: To Study HIV-Capsid-Nuclear Envelope Interactions Leading to Nuclear Import

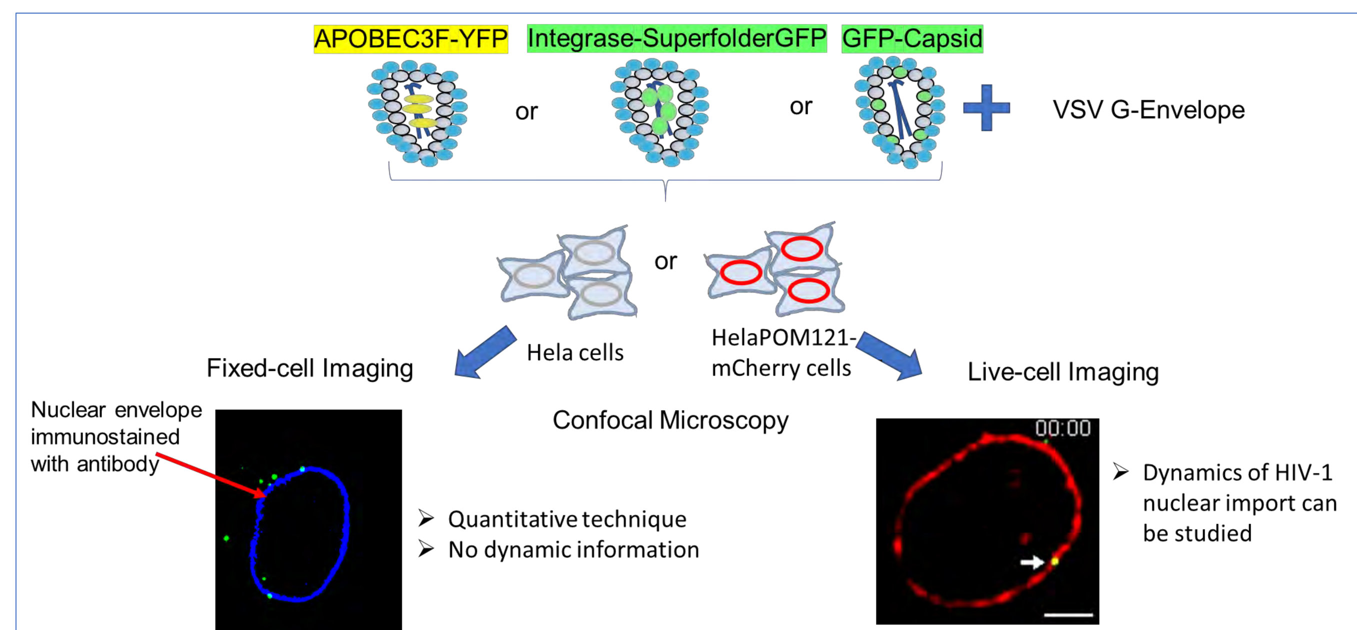
METHODS

CA Mutant	Reported Infectivity (%)	Location/phenotype
M10I	19	Outer surface
M10V	11	Outer surface
I15V	32	Near outer surface
P38A	<5	Hypostable core
E45A	<5	Hyperstable core

Rihn, S. et al. PLoS Path. 2013
Forshey, B. et al. J.Virol. 2002

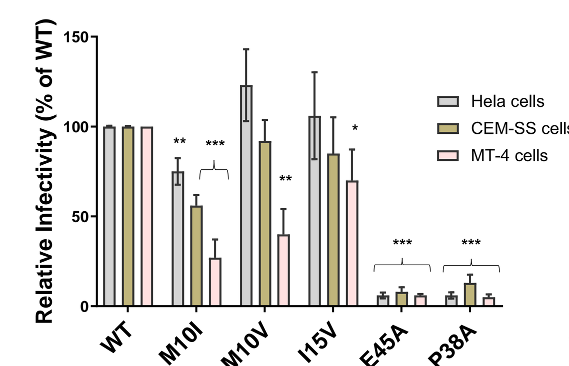


Side view of CA hexamer



RESULTS

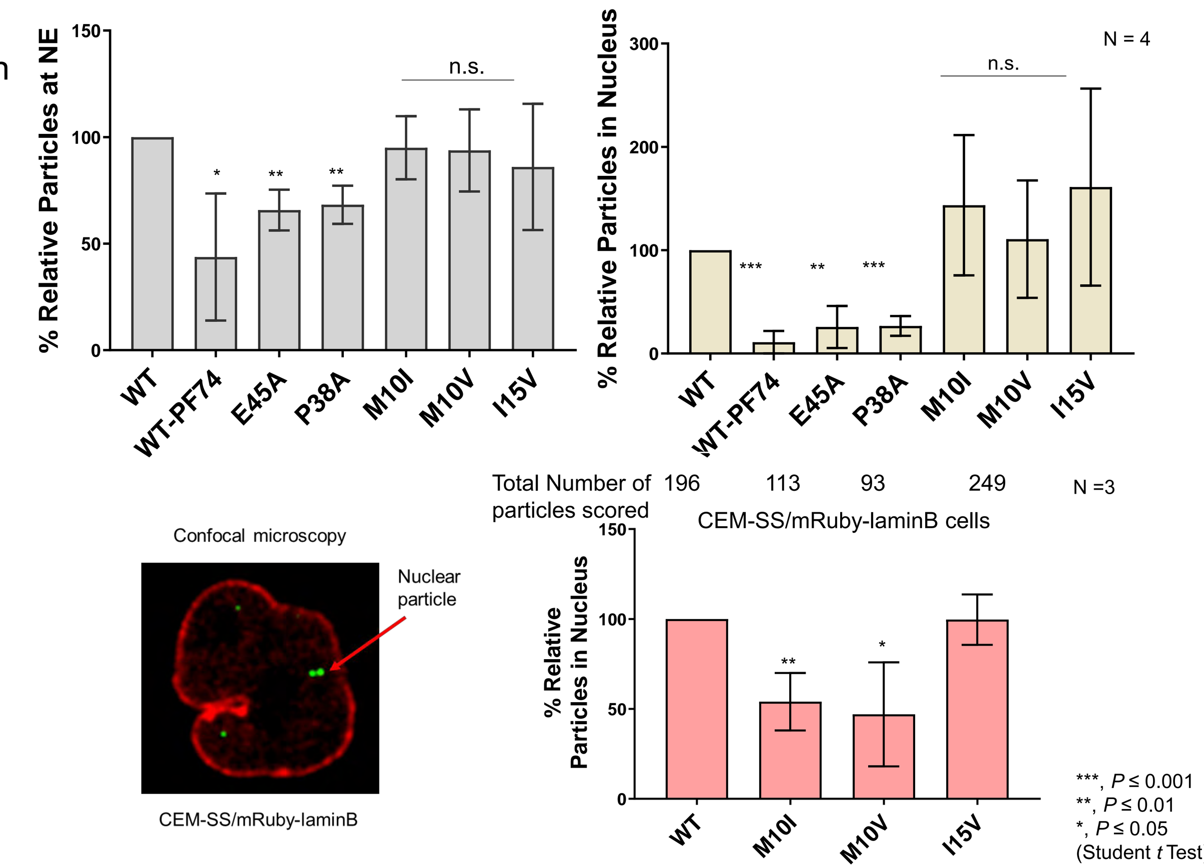
Infectivity



- Hypostable (P38A) and hyperstable (E45A) mutants were defective across all three cell types
- Defects in infectivity of CA mutants were more apparent in MT4 cells

To our knowledge, M10 capsid mutant is the first CA mutant that has been identified to display a CypA dependent phenotype of long NE-residence time for nuclear import.

Fixed-Cell Imaging in HeLa Cells and CEM-SS/mRuby-laminB Cells



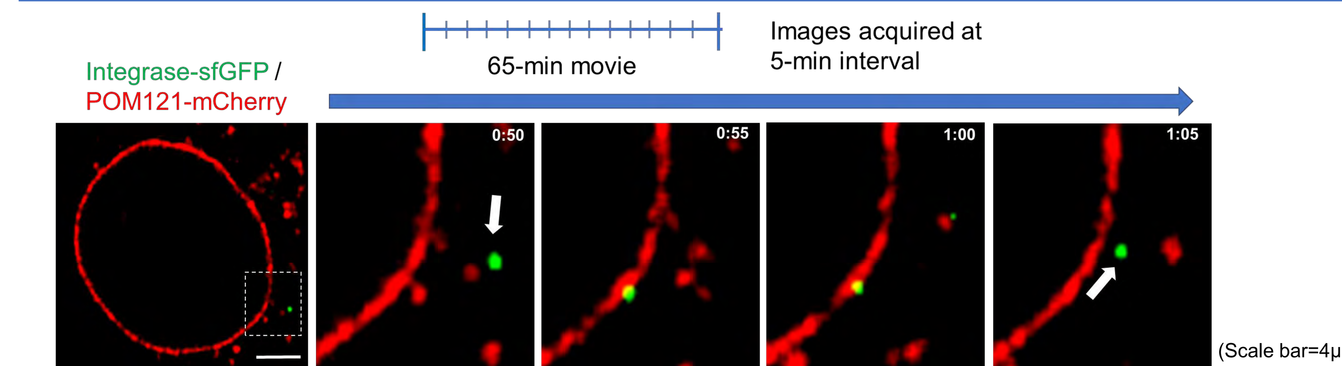
<u>Fixed-cell imaging in HeLa cells;</u>

- Integrase-Superfolder-Green Fluorescent Protein (IN-sfGFP) labelled virus(*Francis et. al.AIDS Res..Hum..Retro.* 2014) were used to infect HeLa cells,6 hrs after infection, cells were fixed and stained with anti-lamin antibody
- Confocal microscopy and image analysis was performed to quantify number of particles at NE and in Nucleus
- Mutants at M10 and I15 residue were similar to WT in terms of NE-association and nuclear import in HeLa cells

<u>Fixed-cell imaging in CEM-SS cells;</u>

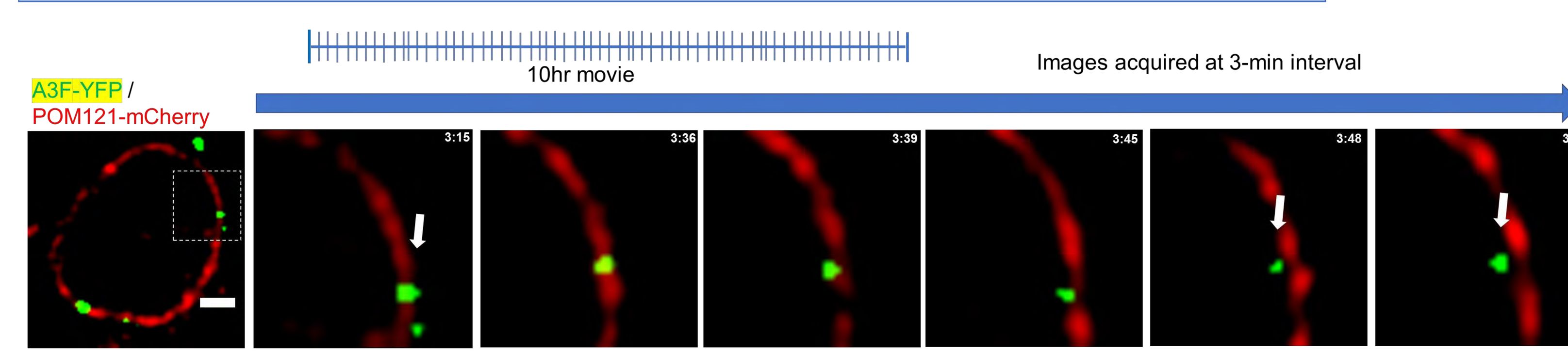
- CEM-SS cells expressing mRuby-laminB were infected with viruses labelled with A3F-YFP, 6 hrs after infection, cells were fixed and confocal microscopy was performed
- M10 mutants exhibited defects in nuclear import in CEM-SS/mRuby-laminB cells similar to their infectivity

Live-cell Imaging For NE-Docking



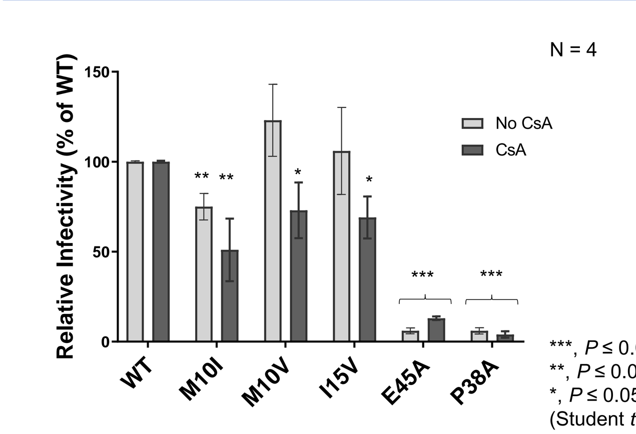
- Most particles that are docked at the NE dissociate from the NE and only a small proportion are imported into the nucleus
- A high-throughput live-cell NE-docking assay was performed in which HeLa-Pom121-mcherry cells were infected with IN-sfGFP labelled viruses and imaged every 5 min for 1-hr
- Mutants M10 and I15 displayed longer NE-docking times than WT

Live-Cell Imaging to Assay Translocation of HIV-1 core though Nuclear Pore Complex



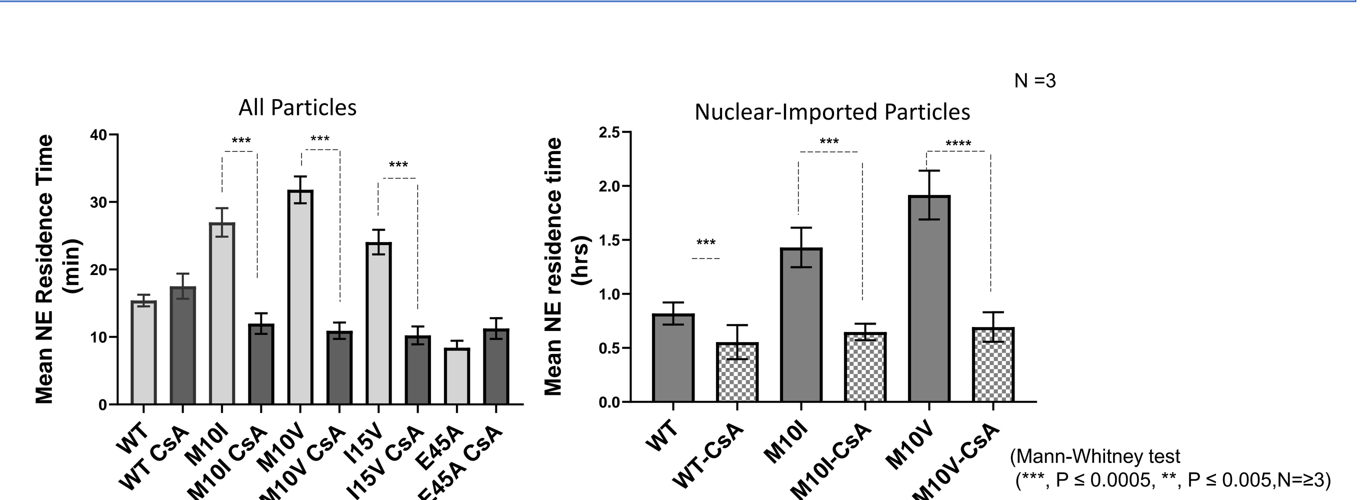
- To determine NE residence of imported particles, HeLa Pom121-mcherry cells were infected with virions labeled with A3F-YFP and live-cell imaging was performed
- M10 mutants displayed longer NE-residence times compared to WT for particles imported into the nucleus

Infectivity in The Presence of CsA in HeLa Cells



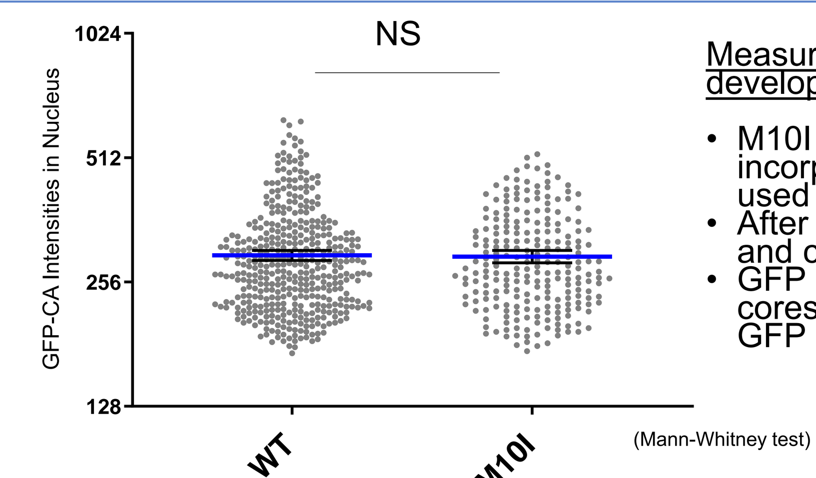
- In the absence of cyclophilin A (Cyp A) binding, HIV-1 virions use a different nuclear import pathway (*Schaller et.al.* 2011) and exhibit a shorter NE residence time (*Burdick et.al. Plos Path.* 2017)
- To determine effect of cyclophilin A on infectivity of the CA mutants, HeLa cells were infected in the presence or absence of 5 µm cyclosporine A (CsA)

NE Residence Times in The Presence of CsA



- To determine effect of cyclophilin A on NE-docking and nuclear import, live-cell movies were captured with and without 5 µm CsA
- In presence of CsA, the mutants exhibited shorter NE-docking times which can be also correlated to the decrease seen in infectivity in HeLa cells
- The NE-translocation times for nuclear imported particles in presence of CsA were similar to WT, suggesting use of similar import pathway

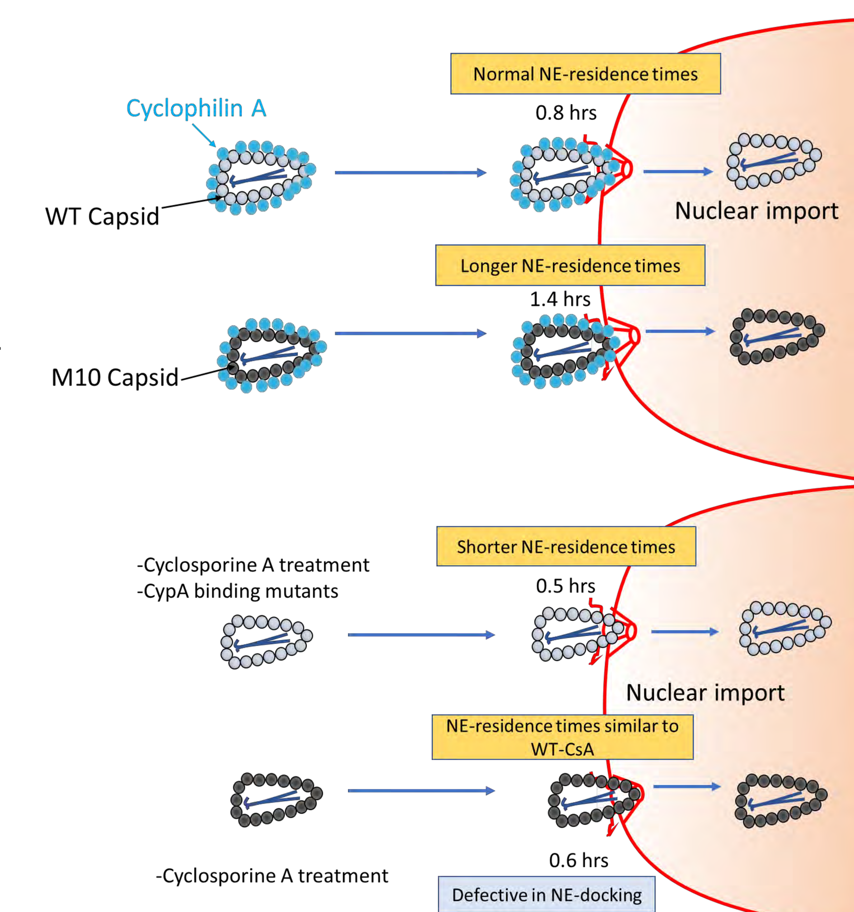
Capsid Levels Inside The Nucleus Using Direct Labelling of Capsid



<u>Measurements of CA levels by using newly developed GFP labelled CA ;</u>

- M10I viral cores having similar GFP-CA incorporated into viral cores as WT were used to infect HeLa cells
- After 6hrs post infection, cells were fixed and confocal microscopy was performed
- GFP intensity of nuclear imported viral cores was determined, M10I had similar GFP intensity to WT

CONCLUSIONS



We have identified CA determinants, which compared to WT exhibit:

- Increased NE-docking time
- Longer NE-residence time for nuclear imported particles
- Defects in NE-docking and infectivity in absence of CypA binding
- Similar NE-translocation times in absence of CypA binding
- Defects in nuclear import and infectivity in T-cell lines
- Lastly M10I mutant has similar CA-levels associated with cores in nucleus

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