Improving the binding affinity of the integrase binding domain in host proteins for HIV-1 virion incorporation

Laila Hayes[1] and Asha Maria Mathew[1, 2]

[1]Department of Biological Sciences, [2]Institute of Molecular Biophysics, Florida State University

Background

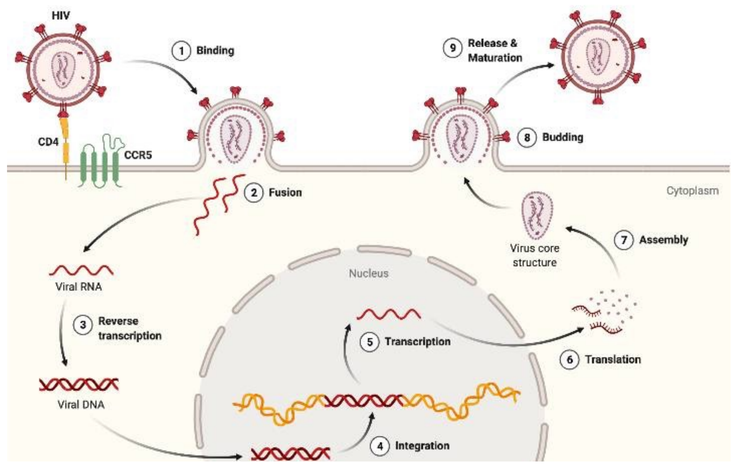

Figure 1: HIV integrates into cellular chromatin and becomes part of host cells Human immunodeficiency virus (HIV) is a lentivirus that integrates its viral cDNA into host cell's genome upon infection. The integrated vDNA becomes a part of the host-chromosomes and is copied to daughter cells after cell division. This property makes HIV-1 an attractive tool in gene-therapy, which aims to incorporate therapeutic genes into sick cells. However, HIV integration occurs throughout the entire human genome, which poses potentially detrimental effects[1]. Controlling the location at which integration occurs is needed to use HIV-based vectors for gene therapy applications.

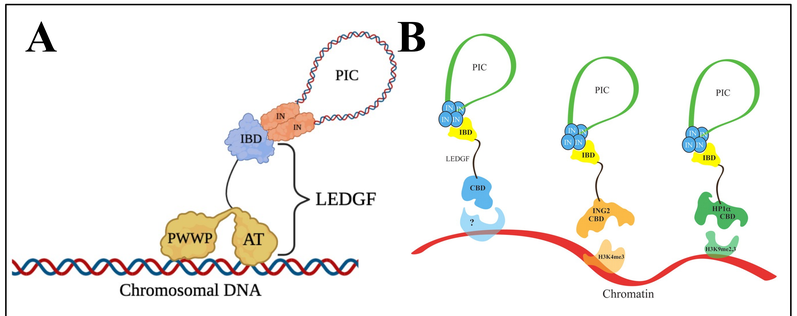

Figure 2: LEDGF tethers integrase to cellular chromatin - A) HIV integration is catalyzed by the viral enzyme integrase (IN), which modifies vDNA strands for insertion into host chromatin. IN requires the aid of the lens epithelium-derived growth factor (LEDGF), a protein that tethers IN to cellular chromatin[2]. LEDGF has two specialized domains for this function; at the C-terminal region, the protein has an integrase binding domain (IBD) that connects to IN in the pre-integration complex (PIC). The N-terminal region has a PWWP domain and an A&T hook, which bind to cellular chromatin and control where integration occurs in the host genome[3]. **B)** Replacing the N-terminus structures with alternative chromatin tethers has been shown to redirect integration at the epigenetic level[4]. These proof-of-concept experiments indicate alternative tethers can be attached for single-site targeted integration.

Current Limitations - Fusion proteins with CRISPR/Cas9 and IBD have been engineered to mimic LEDGF in tethering IN to a specific chromatin site. However, these proteins exhibited a low binding affinity to IN, showing poor incorporation into HIV virions and limiting their practical use.

Experimental Approach

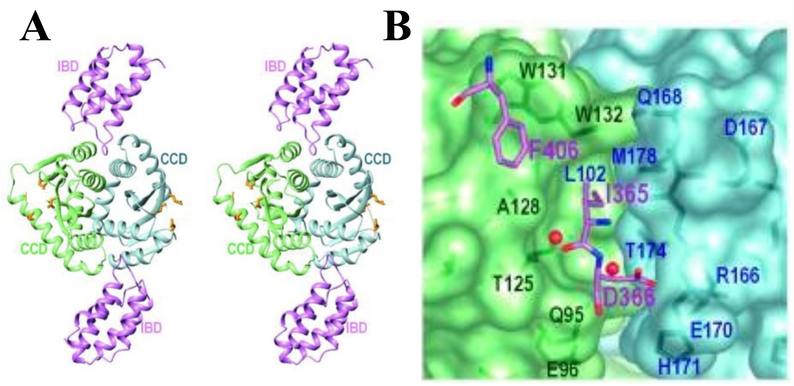

Figure 3: Molecular mechanism of IBD-CCD complex interaction - A) X-ray crystal structures show that the IBD binds to the catalytic core domain (CCD) of IN, creating an IBD-CCD complex. IN helixes (CCD) are in blue and green, and the IBD is in violet. **B)** Intermolecular interactions between IN and IBD amino acid residues maintain the complex's strength. LEDGF residue Ile-365 creates hydrogen bonds with IN's Thr-125, and the hydrophobic side chain of Ile-365 burrows into the hydrophobic pocket formed by IN residues Met-178, Leu-102, Ala-128, Trp-174.[5]

Experimental Approach - Increasing the hydrophobicity of IBD residues strengthens the IBD-CCD complex. In this project, I will develop an I365C IBD mutation protein, analyze its incorporation into virions analyzed using single-virus imaging and analyze its infection via infectivity assays.

Preliminary Results

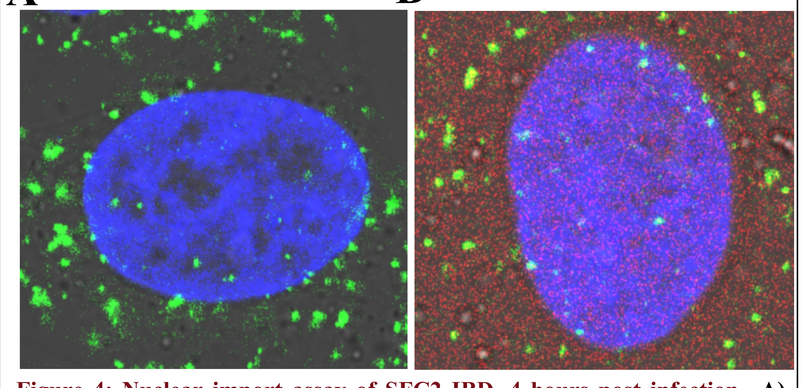

Figure 4: Nuclear import assay of SFC2 IBD, 4 hours post infection– A) TZMbl cell were infected with INmNG HIV-1 virus. **B)** TZMbl cell were infected with SFC2 IBD INmNG HIV-1 virus with the IBD particles in red. Yellow overlap shows the IBD-IN binding, with successful nuclear import of HIV. Similar images will be collected for the 1365C IBD.

Preliminary Results Continued

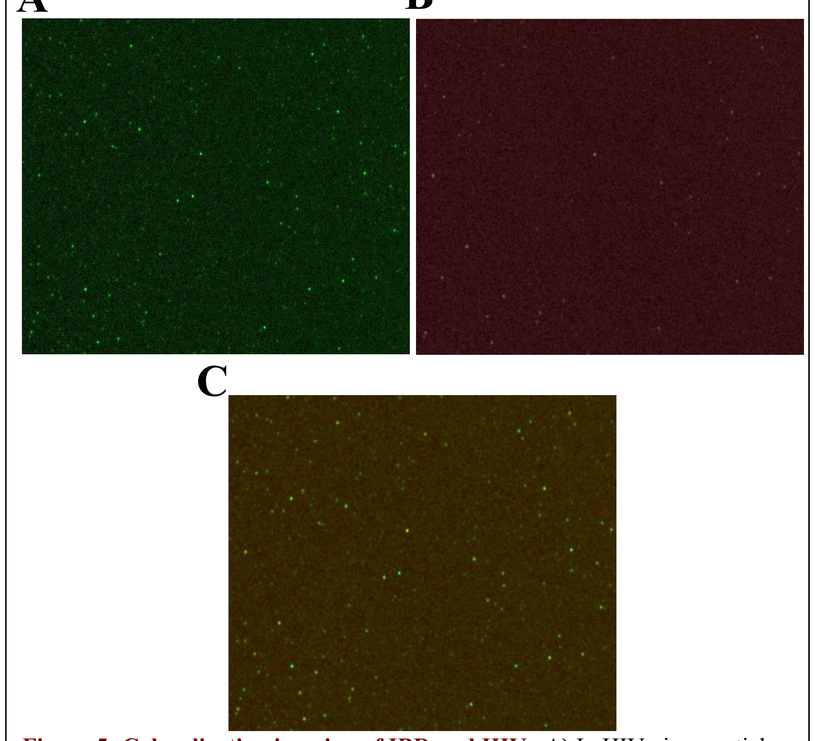

Figure 5: Colocalization imaging of IBD and HIV– A) In HIV virus particles, integrase is tagged with mNeonGreen. **B)** In the same particles, IBD is labeled with SFC2. **C)** Overlaying images A and B shows how effectively unmutated IBD binds to IN. Similar images will be collected for comparison of interactions between the 1365C IBD mutant and IN.

References

1. GW School of Medicine & Health Science. (n. d.) HIV Infection and Aids. https://bosquepardoslab.smhs.gwu.edu/research-interest
2. Craigie, R. (2010). Targeting HIV-1 DNA integration by swapping tethers. *Proceedings of the National Academy of Sciences, 107*(7), 2735-2736. https://doi.org/10.1073/pnas.0915097107
3. , S. & Cherepanov, P. (2009). The interaction between lentiviral integrase and LEDGF: Structural and functional insights. *Viruses, 1*(3), 780–801. https://doi.org/10.3390/V1030780
4. Engelman, A., & Cherepanov, P. (2008). The lentiviral integrase binding protein LEDGF/p75 and HIV-1 replication. *PLOS pathogens, 4*(3), *e1000046.* https://doi.org/10.1371/journal.ppat.1000046
5. Meehan, A. M., Saenz, D. T., Morrison, J. H., Garcia-Rivera, J. A., Peretz, M., Llano, M., Poeschla, E. (2009). LEDGF/p75 proteins with alternative chromatin tethers *are functional* HIV-1 cofactors. *PLOS Pathogens 5(7), e1000522. https://doi.org/10.1371/journal.ppat.1000522*