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Analyzing Conformational Changes in the Binding of HIV-1 Matrix Protein, N and C Terminals, to Calmodulin

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Investigating the Interaction of Calmodulin N and C Terminals with the Hiv-1 Matrix Protein Peptide

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Introduction:

Human immunodeficiency virus (HIV) type 1 is the most common type of HIV worldwide. Initially, the virus attaches itself to a CD4 cell and takes control of its DNA and machinery through its fusion to the cell; copies of the virus itself are then made and released into the bloodstream of the infected individual. HIV-1 has a group specific antigen, the Gag protein, with the HIV matrix protein component. This essential matrix protein (MA) is composed of a myristyl group on its N-terminal that targets the plasma membrane, allowing for the continuation of its viral replication. Calmodulin (CaM) is a calcium-binding and calcium-modulated protein located in the cytoplasm of eukaryotic cells. Calmodulin has an N-terminal domain and a C-terminal domain, the latter of which functions in the absence of calcium while the N-terminal does not. See figure 1. These domains undergo conformational changes between an open and closed form, in order to expose their hydrophobic cores where their target-binding globular domains reside. These binding affects are hypothesized to facilitate increased viral production of HIV-1 but also display that the hindering of the matrix protein binding site could lead to reduced viral replication.

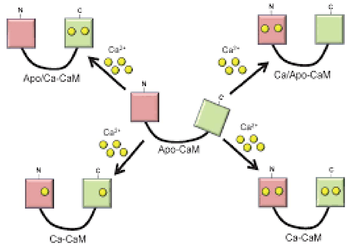


Figure 1: *Binding of Ca^{2+} to calmodulin in APO and Calcium conditions (Papoff, 2015)*

Methods:

Fluorescence Spectroscopy - Analyzes fluorescence of a molecule based on fluorescent properties. Photons excite the molecule, raising it to an electronic excited state, causing luminescence. Exciting the molecule's electrons causes them to emit light and that light is taken to a filter and to a detector in which measures changes in the molecule. Through the data collected with florescence anisotropy, detailed information about the binding tendencies of CaM with full MA, N-terminal MA, and C-terminal MA in both APO and Ca^{2+} conditions will be revealed.

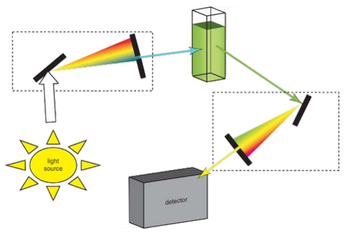
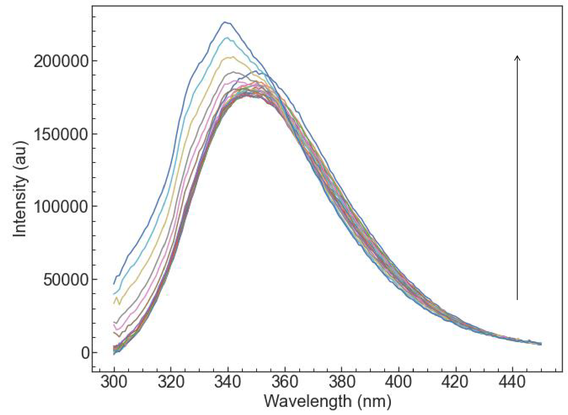


Figure 2: Mechanism of Fluorescence Spectroscopy

References:
Papoff G. et al. N-Terminal and C-Terminal Domains of Calmodulin Mediate FDD and TRADD Interaction. National Library of Medicine (2015); 10(2): e0116251.

Results:

C-Terminal MA and CaM in Ca^{2+} conditions



C-Terminal MA and CaM in APO conditions

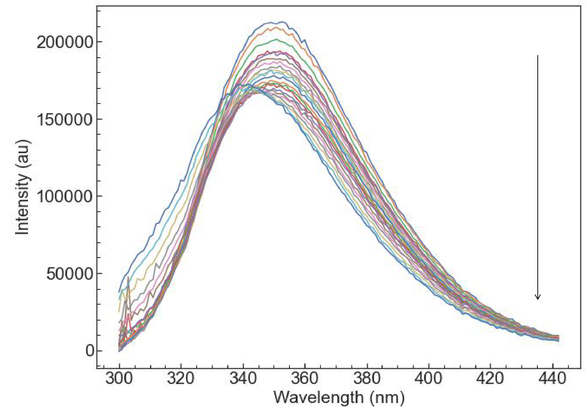
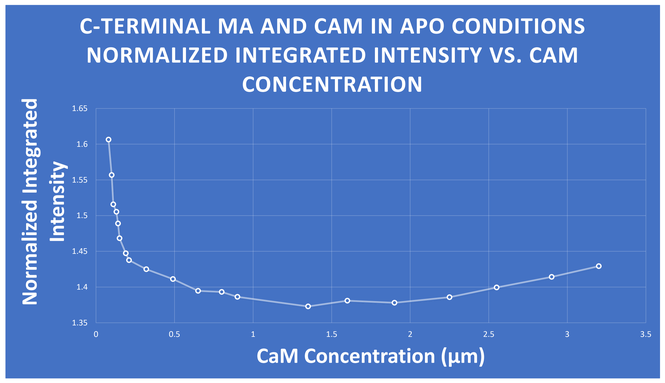


Figure 3: Fluorescence data for trials C terminal HIV-1 Matrix Protein titrated with C terminal Calmodulin in both Ca^{2+} (left) and APO (right) conditions, collected with fluorometer. Arrows depict direction of progressing titrations. (Karimi, 2022)



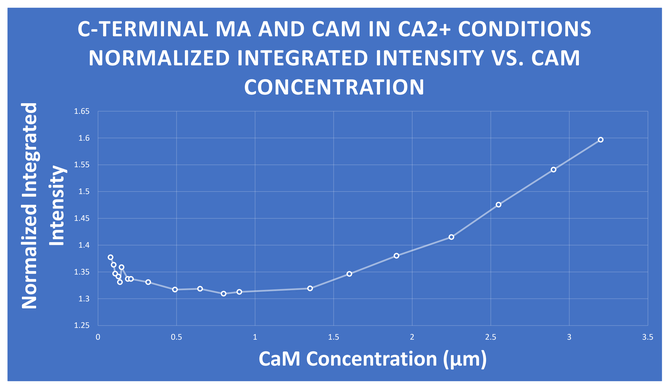


Figure 4: Titration curve of C terminal HIV-1 Matrix Protein titrated with C terminal Calmodulin in both APO (left) and Ca2+ (right) condition, collected with fluorometer. Integrated data depicting how titration evolved over time. (Karimi, 2023)

Conclusion:

Findings - In the data above, there were shown to be no binding interactions between the MA protein and CaM under APO conditions, which further solidifies our hypothesis that CaM and the MA protein bind in a Ca^{2+} dependent manner; this is evident by the lack of blue shift in APO conditions as well as increase in intensity. A blue shift would indicate that there is a conformational change occurring, and therefore binding occurring.

Conclusion – The data indicates that in conditions without calcium, the HIV MA protein will not bind, leading to the potential disruption of the HIV replication cycle. The data shown above is in the context of a larger data set that includes N-terminal and FULL protein binding interactions. From all data collected, neither N-terminal, C-terminal, or FULL MA with CaM in APO conditions show significant protein binding. There was initial non-specific rapid quenching present in both APO and Ca^{2+} trials of N-terminal MA and CaM binding, which indicate that there could potentially be a 2-step binding process occurring. The results of all data collected confirm the calcium dependency of the CaM and HIV-1 MA interaction and further suggests the C-terminal role as a pre-requisite for N-terminal MA binding.

Future Work - In our data from this past semester, we were able to collect cohesive and consistent data to adjust past student errors in procedure and have begun to collect solid reproducible data to publish. We were, and continue to be, cautious with procedural methods, precision, and cleanliness in creating buffer, protein solutions, and throughout titrations.