

Response of Erythrovirus B19 Capsid Protein VP1up to Small Molecules as Observed by SAXS and SRCD. Keywords: erythrovirus, PLA₂, calcium, SAXS, SRCD

My interest in viruses originated in the undergraduate classroom of Michael Hadjiargyrou, a professor of biomedical engineering. During these lectures I was introduced to gene therapy, in which a viral vector could be used to deliver curative transgenes. This has been used to confer benefits to patient in the form of therapeutic protein expression.

After expressing my interest in virology to my mentor, Marc Allaire of the NSLS, I was assigned the crystallography project described which has expanded to include the investigation of calcium on VP1up structure. These perturbations were accessed by synchrotron radiation circular dichroism (SRCD), an established spectroscopic method for analyzing the secondary structure of proteins. Measurements in the far UV wavelength range provide structural information about backbone conformations of a polypeptide in solution. This can discern between properly folded proteins from unfolded ones. Even small changes in conformation often indicate a change in the ordered state of the protein which cannot perform its function. We hope to take advantage of this by testing the small molecule inhibitors on pathological proteins from viruses. Our goal is to establish new, efficacious, publicly available assays for the discovery of pharmacological ligands which could treat disease and be used for more functional studies. The proposed assays harness both high energy x-ray and UV radiation available at the NSLS.

SRCD is an emerging technique available at six beamlines worldwide, two of which are located in the U.S., both at the NSLS. Conventional CD is found in many labs; however SRCD provides higher flux allowing the user to explore the vacuum ultraviolet region (VUV) unavailable on bench-top equipment. This region contains additional electronic transitions

adding to the information content of a waveform. Little is known about the structural contributions below 190 nm as data is scarce. This is due to the low signal-to-noise ratio of bench-top CD machines in the VUV which is improved by nitrogen purging and UV transparent calcium fluoride cells at the beamline.

Figure 3 is the result of 24 averaged scans performed at U11 of B19 VP1up with and without calcium. It was

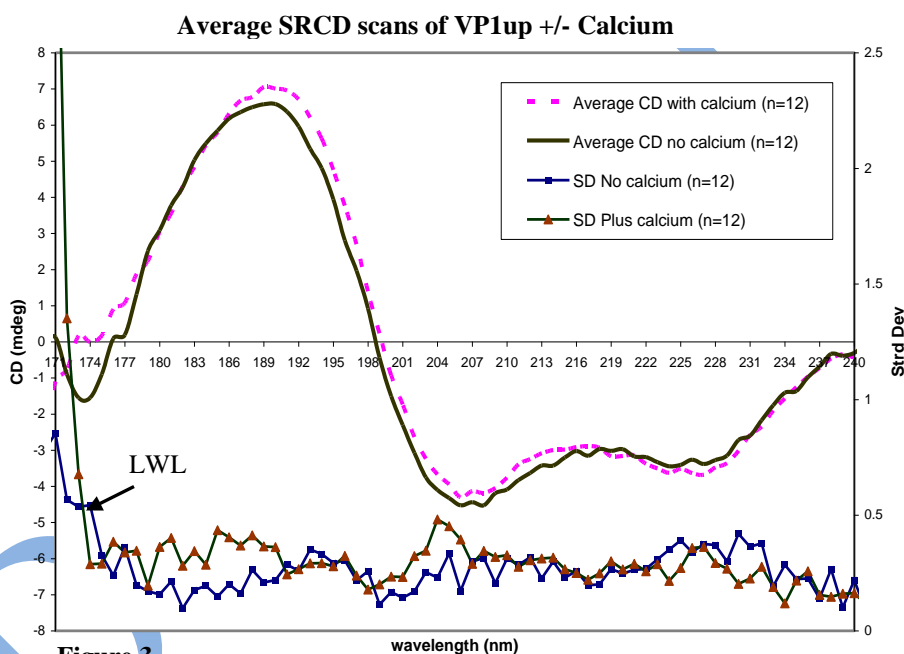


Figure 3

predicted that the presence of calcium would cause a broad structural change in VP1. Previously, it has been demonstrated that the parvoviral PLA₂ domain is active only in the presence of calcium ions which permits entry of the virus in the host nucleus¹. There are only small differences in the two spectra above 178 nm primarily centered on the positive peak at 190 nm caused by alpha-helical proteins². The low wavelength limit (LWL) in this experiment was

determined to be 173 nm, as standard deviation (SD) between spectra rose above their baseline of 0.5σ . Comparisons show minute, yet reproducible fluctuations in VUV CD as demonstrated by the slope of the waveform. At this point it is difficult to say if calcium impacts secondary structure. The LWL can also be defined by the voltage over the photoelastic modulator at U11. Bioinformatics analyses by DICHROWEB³ produced variable results as a function of algorithm and reference set used.

In the literature, there are other more dramatic examples of conformational change caused by point mutation or ligand binding. Landmark examples include HIV gp41 which

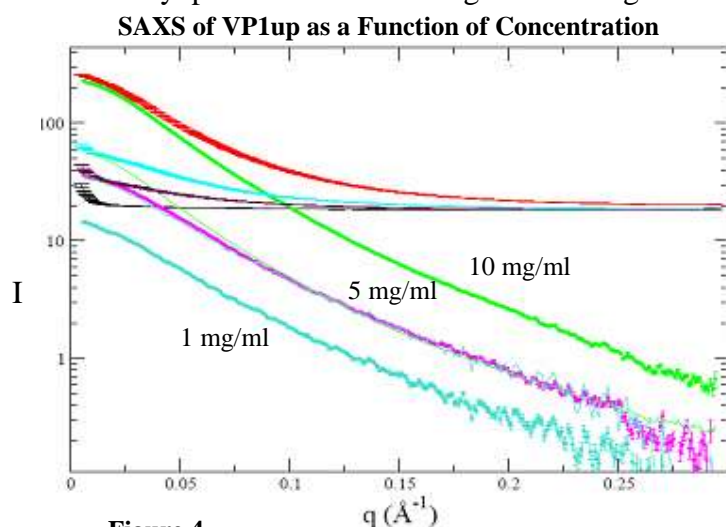


Figure 4

exhibits dramatic disorder of helices when mutated at specific residues important for protein-protein interactions within the envelope⁴. When placed in solution with fusion inhibitor peptides, gp41 also undergoes a conformational shift as evidenced by CD⁵.

¹ Ligands do not have to bind to the active site of a protein to cause structural rearrangement as demonstrated by SAXS or SRCD. This is still very useful for studying the intramolecular effects between proteins. For example, a conformational change that does not inhibit the function of

VP1up *in vitro* may still prevent B19 erythrovirus infection when studied in the context of an intact capsid. SAXS of proteins in solution provides several advantages over crystallography and SRCD in terms of HTS. Primarily, the protein is in a dilute solution, opposed to the crystal form. Another major factor is that the time required for SAXS data collection is orders of magnitude less than SRCD. These features make SAXS more agreeable to HTS methodology; however there are problems in the design of sample chambers which could hold hundreds of conditions. Currently, the solution of interest is propelled through a capillary in front of the beam. For HTS, an x-ray transparent 96-well plate would be more advantageous.

An example of SAXS is shown in figure 4. In this picture, the intensity of diffracted x-rays are directly proportional to the concentration of B19 VP1up. The proposed experiment will utilize B19 PLA₂ and bovine PLA₂ to search for backbone remodeling in the presence of a known, commercially available inhibitor for bovine PLA₂, M 3315. This 499 Da substrate mimic binds to the active site as demonstrated by the crystal structure⁶. This complex will be a positive control as we are hypothesizing that a detectable conformational change will occur. The negative control will be B1552, a tight binding inhibitor of other PLA₂ isoforms, but not Type I.

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