

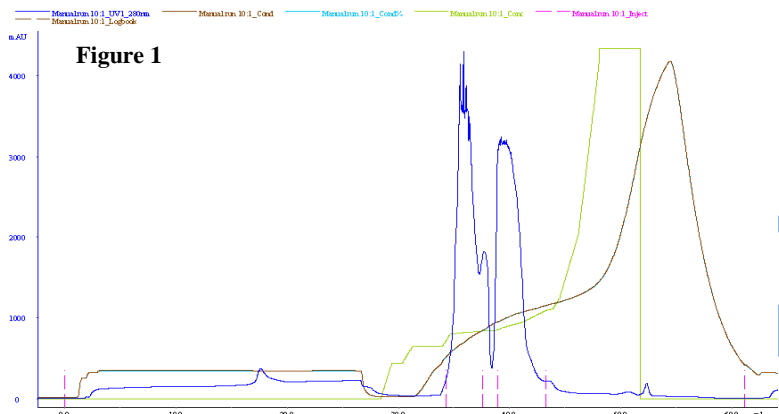
Brookhaven National Labs (BNL) and Stony Brook University have provided me with over four years of graduate and undergraduate research experience. BNL specifically has supplied me with extensive access to world class equipment found in few other research settings. Most importantly, my employment has allowed me to meet renowned international scientists and connected me with a fantastic mentor. Originally hired as an undergraduate summer intern, I have continued my work over the last two years independently purifying several different viral proteins from erythrovirus B19, porcine parvovirus, and hepatitis C virus. The focus of this application will be on the erythrovirus B19 capsid protein which I have characterized by high pressure liquid chromatography (HPLC), MALDI-TOF mass spectrometry (MS), synchrotron radiation circular dichroism (SRCD), and small-angle x-ray scattering (SAXS).

My undergraduate research capabilities have quickly transitioned into skills necessary for graduate level work. As a graduate student I have taken on the responsibility of training and teaching a new member of our lab. In a few months time, she was producing excellent results with MS and HPLC. Teaching has been an extremely rewarding experience, as my colleagues can now complete projects independently. After sharing results, we mold experiments together in order to maximize learning opportunities and shared knowledge.

The focus of my research has been on the crystallization of a domain from the human parvovirus B19 (B19) capsid responsible for the infection of human erythrocytes. B19 is an infectious pathogen frequently contracted by immunologically and hematologically compromised patients. Routes of transmission include blood transfusion, respiratory infection or vertical transfer. Clinical manifestations include erythema infectiosum, anemia, spontaneous abortion and polyarthropathy. Patients with underlying blood disorders undergo transient aplastic crisis due to the viral tropism for erythroid progenitor cells of the bone marrow. B19 tropism is correlated with the presence of glycolipid globoside, a blood group P antigen. Patients lacking this receptor are immune to B19. Infected individuals experience hemolysis, severely afflicting those with sickle cell, autoimmune hemolytic anemia, and HIV. Immunodeficient patients unable to generate IgM and IgG antibodies experience persistent viremia. The amino terminus of viral protein 1 (VP1) is the principal antigenic site. The method of successful antibody neutralization is unknown, but is probably due to a conformational change in the capsid or its receptor inducing steric hindrance at the binding site.

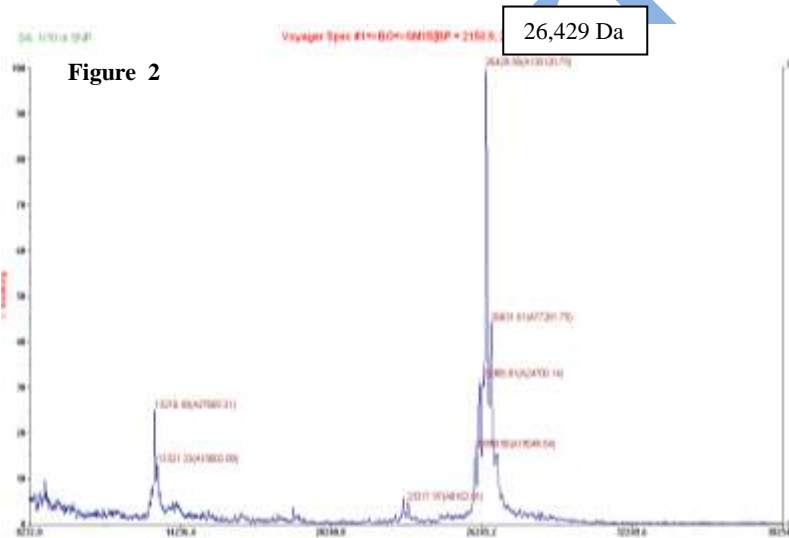
The linear double stranded DNA genome is encased by the capsid consisting of two structural proteins, VP1 and VP2 packed in a 1:5 (VP1:VP2) ratio. This arrangement creates large grooves circumscribing prominent cylindrical pores at each five-fold axis. Residues at the foot of these depressions are responsible for receptor attachment and are sheltered by the canyon rim thwarting antibody recognition. VP1 and VP2 are identical excluding the VP1 unique portion (VP1up) which extends 227 amino acids past the amino-terminus of VP2. Enzymatic analysis, mutagenesis and immunofluorescent confocal microscopy studies performed by our collaborators identified a calcium-dependent phospholipase A₂ (PLA₂) domain on VP1up functionally homologous to cytosolic PLA₂. PLA₂ is a lipolytic enzyme which hydrolyzes the *sn*-2 ester-linkage of phospholipids liberating fatty acids and lysophospholipids from their glycerol backbone. It is hypothesized that upon entering the cell, this portion of the capsid withstands calcium induced conformational changes exposing catalytic residues that hydrolyze phospholipids at the nuclear membrane. Point mutations within the catalytic dyad (His, Asp) inhibit nuclear entry but do not prevent access into the cell.

VP1up was supplied by Peter Tijssen from the Université du Quebec, INRS Institut Armand – Frappier. VP1up protein was extensively purified by HPLC for crystallization. This procedure utilizes Ni ion affinity, desalting, anion exchange, and gel filtration chromatography to separate protein mixtures. After lysing *E. Coli*, the his-tagged protein is applied to a nickel column and eluted with 1M NaCl and 250mM imidazole to remove soluble proteins. The single peak is digested by TEV protease at two sites, flanking the protein of interest removing the N-terminus thioredoxin and C-terminal his₆-tag. The fragments in solution are desalted and applied to the Ni-column again to remove the protease. A second desalting is required for anion exchange chromatography. VP1up (pI=6.5) is kept in 20mM Tris pH 8.5 allowing it to stick to



the positively charged MonoQ column, on which it is separated from thioredoxin by a NaCl gradient (figure 1). To eliminate contamination by the digested products, the sample is concentrated and injected onto a gel filtration column with 200mM NaCl. This method consistently yields 14% recovery (n=15) in which VP1up can be concentrated to 50 mg/ml.

After elution from the MonoQ and gel filtration columns, peaks containing VP1up are analyzed by mass spectrometry to verify digestion and homogeneity. Results in figure 2 illustrate the VP1 molecular weight equal to 26,429 Daltons, corresponding to the 227 residue protein product. After concentration, the protein is suitable for crystallization screening.



coordinating activity in the active site slot. Experiments that included 2.5mM calcium in the final wash buffer during concentration produced salt crystals. New approaches will add lower calcium concentrations to the purification buffers. After binding, it is thought that excess calcium could be removed by a wash on the gel filtration column. Regional presentations and publications: New York Structural Biology Discussion Group (NYSBDG) summer meeting 2006, NYSBDG winter meeting 2005, U.S. Department of Energy Journal of Undergraduate Research Summer 2005, SBU Hospital Department of Anesthesiology Research Awareness Series Summer 2006/2005, City College of New York Einstein's in the City Conference Spring 2005, SBU Hospital Department of Anesthesiology Department Research Seminar Fall 2004.