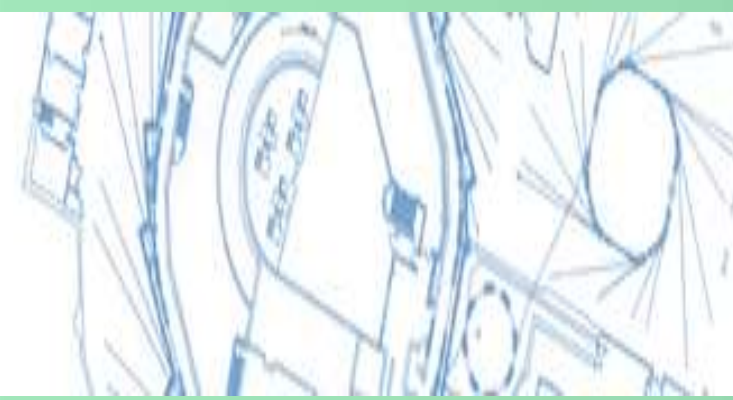


# Purification of Parvoviral B19 Phospholipase A<sub>2</sub>



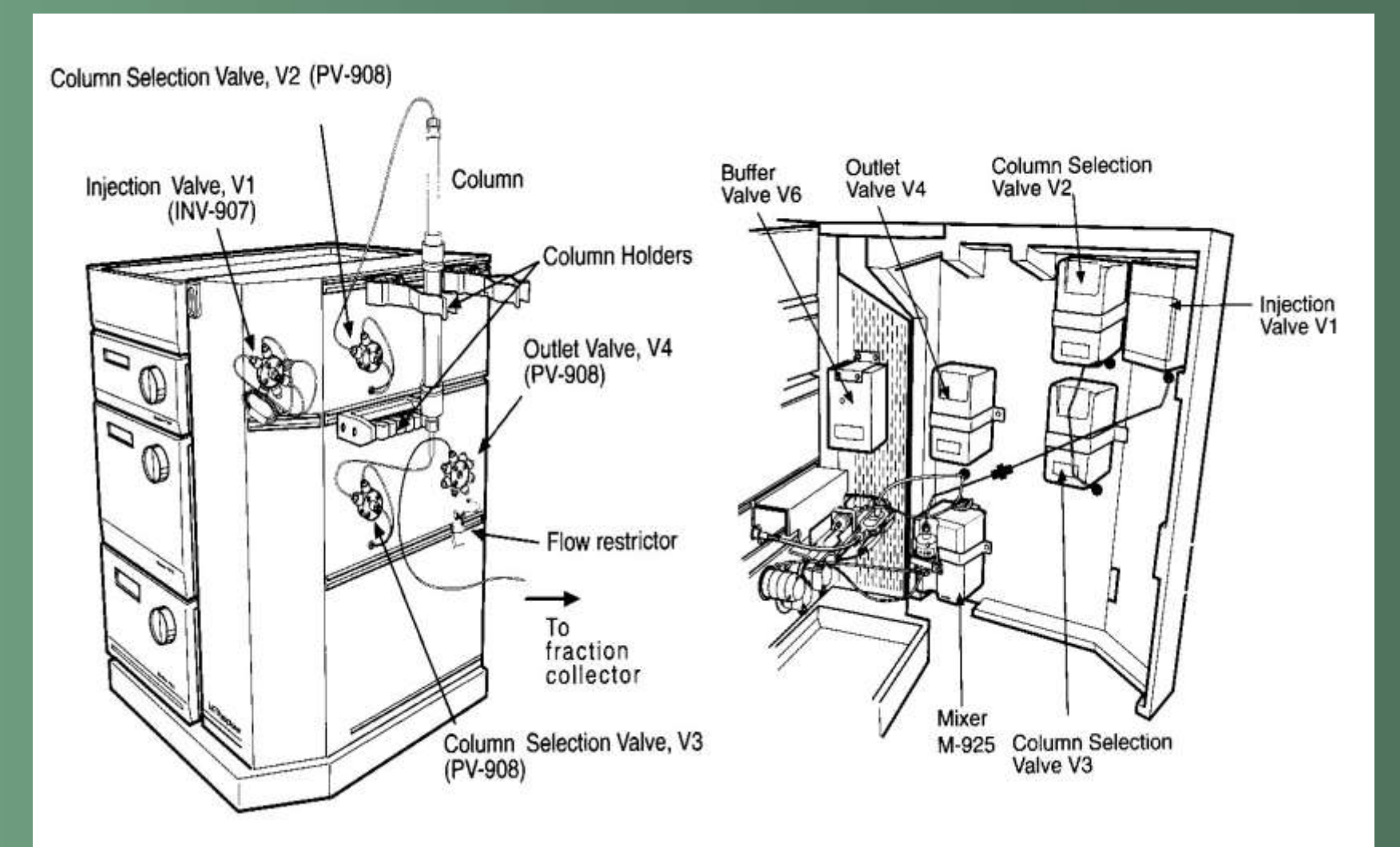
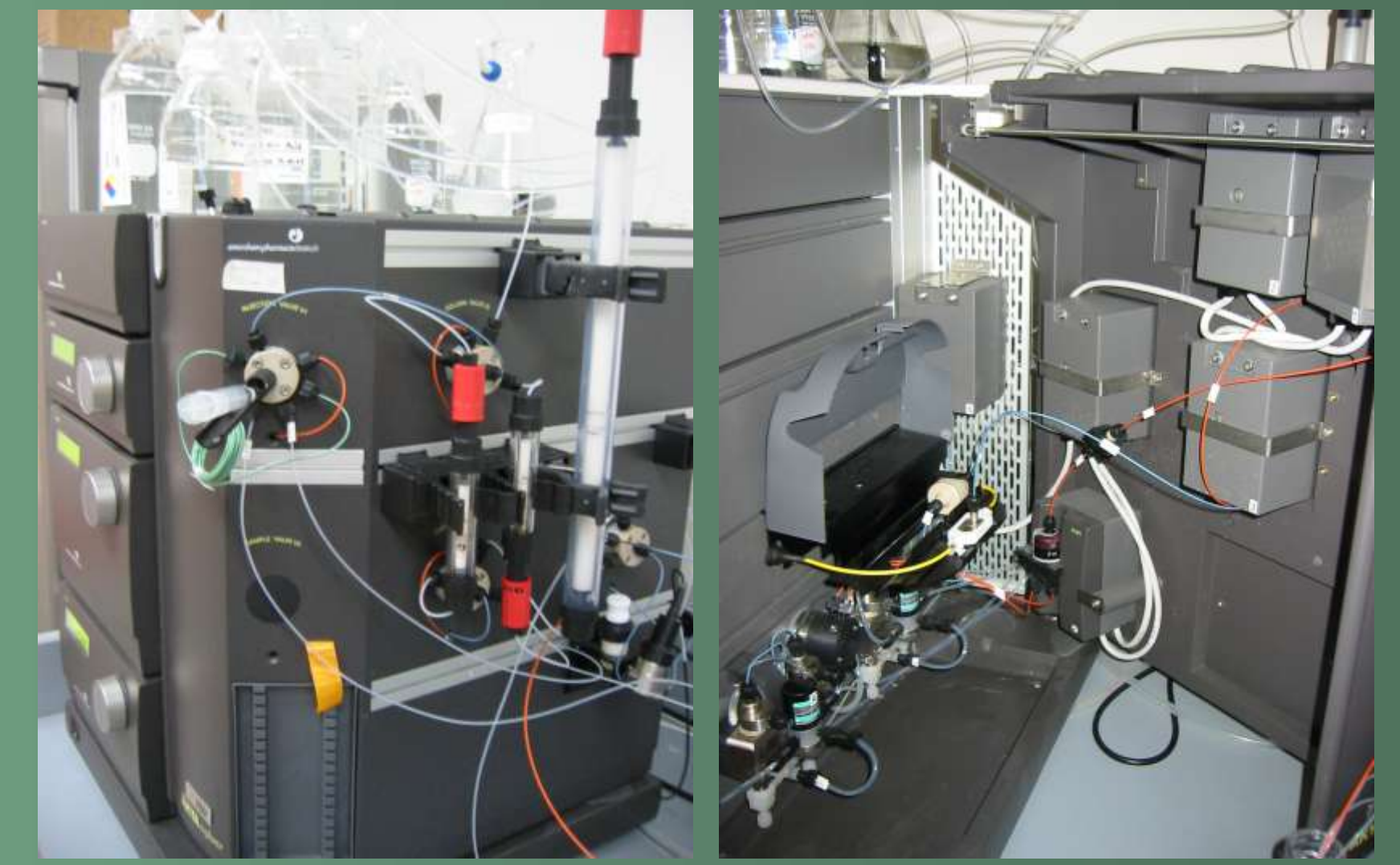
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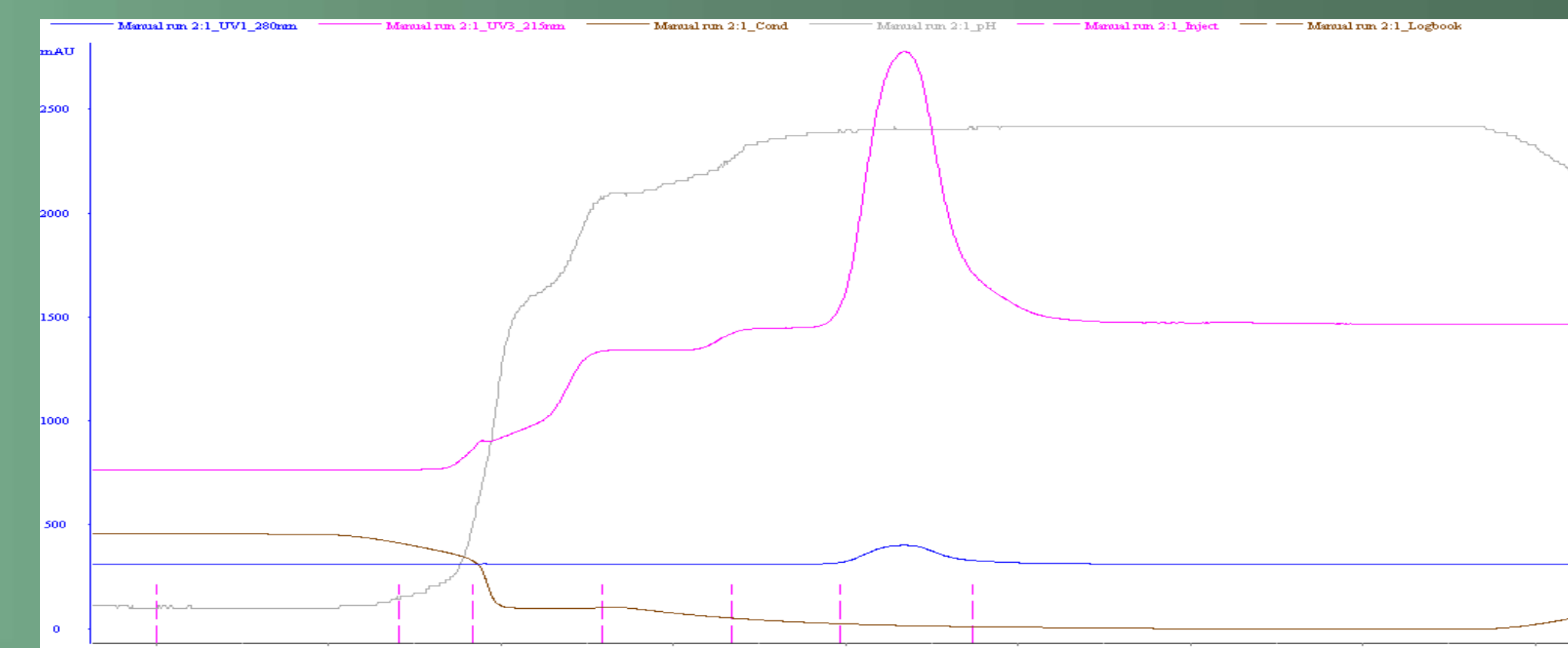
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## Background

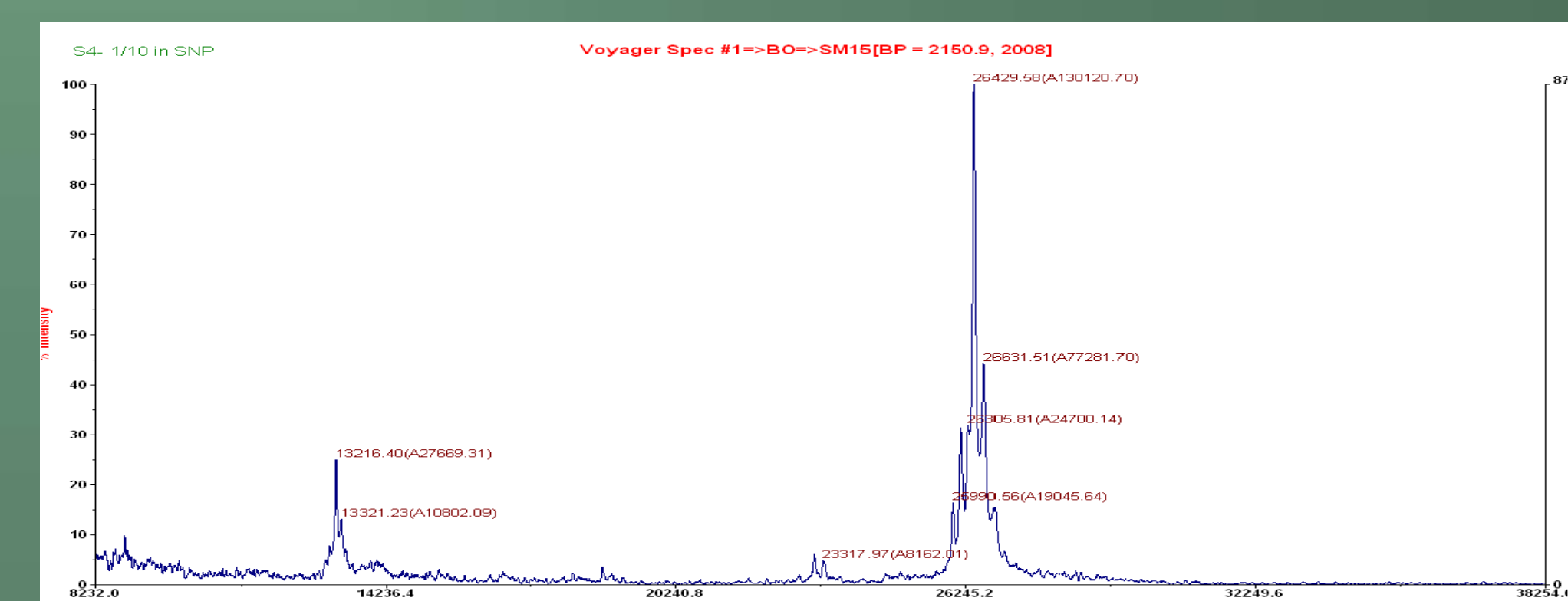
The human parvovirus B19 is a 5.6 kb single-stranded DNA containing virus. Its structure and genetics are similar to other parvoviruses, including the adeno-associated virus (AAV), a promising vector for gene therapy. The genetic material of B19 is contained in the capsid which governs infectivity, receptor binding, immune response, host cell range and tissue tropism. The capsid of B19 is composed of two structural proteins named VP1 and VP2. VP1 contains the 554 amino acids (aa) of VP2 but stretches for an additional 227 aa at the N-terminus. This unique overhanging portion (VP1up) exhibits phospholipase A<sub>2</sub> (PLA<sub>2</sub>) activity and is essential for infectivity. To further understand the PLA<sub>2</sub> interfacial enzymology and its role in the viral life cycle, a high resolution three-dimensional structure is necessary. In collaboration with Z. Zádori and P. Tijssen (INRS-Institut Armand-Frappier, Laval, QC Canada), the PLA<sub>2</sub> domain of B19 was expressed in *E.Coli* as a fusion protein flanked by thioredoxin and a histidine tag. The 399 aa fusion protein was digested to remove these fragments and the region of interest was purified with high performance liquid chromatography (HPLC). This method relies on intrinsic biochemical and physical properties of the protein fragments for separation. The purification method consisted of anion exchange chromatography followed by gel filtration. The size of VP1up was verified by mass spectroscopy during HPLC to correlate each fraction with its corresponding fragment. Results have yielded samples pure enough for crystallization studies of the B19 PLA<sub>2</sub> domain. We expect the high resolution atomic structure of VP1up will contribute to the further understanding of B19 PLA<sub>2</sub> enzymology and its role in infectivity.



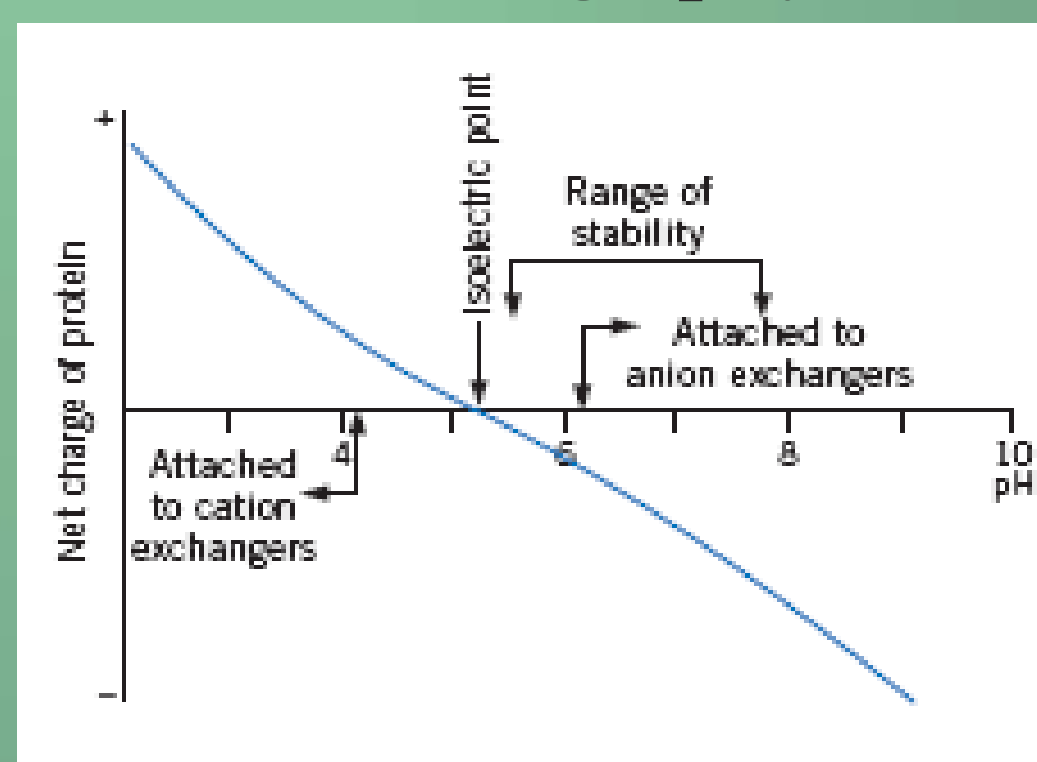
The above diagram is of a high performance liquid chromatography machine known as the ÄKTA explorer designed by GE Biosciences. Chromatography is a technique used to separate proteins based on their interaction with a matrix. Due to the high selectivity of each column, purification can be completed in steps with high resolution and concentration. Electrostatic or hydrophobic relationships between the target molecule and ligand induce retention within the beads of a matrix.



Above is the polished B19 fragment from peak two (Figure A) flown through a Superdex 75 gel filtration column. The disadvantages of this column are its small sample volume and slow time. However, it provides extremely high purity as verified below by the mass spectroscopy diagram of the fraction from 8-9.5ml. The 13kD fragment is most likely an ionized form of the B19 peptide.



## Ion Exchange Chromatography



The isoelectric point (pI) of a protein is the value at which the charge equals 0. Amphoteric groups contribute + or - charge dependent on pH. At a certain pH value an amphoteric substance will have 0 net charge- this is the pI. At this point the substance will bind to neither anion or cation exchangers. The experimenter can control the behavior of a sample by choosing a buffer with specific pH causing it to temporarily bind to a column. Adding NaCl causes the sample to elute by increasing the conductance thereby removing the electrostatic bonds.

## Results

The results of this protein purification experiment were verified by mass spectrometry. This allowed us to determine the precise molecular weight of the peptides derived from enzymatic cleavage and HPLC. MALDI-TOF (matrix-assisted laser desorption ionization-time-of-flight) spectrometry ionizes the peptide fragments into a gas where they are accelerated through an electric field and caught on a detector. The 237 residue B19 fragment weighs 26 kilodaltons and appears most pure in figure C which was produced by ion exchange and gel filtration column chromatography. The 13kD fragment is most likely an ionized form of the B19 peptide. The next logical step it to begin crystallization trials in an attempt to solve the structure of this capsid PLA<sub>2</sub> domain. Key conserved domains are expected to resemble the structure of secreted or cystolic PLAs at the active sites. If accomplished this would be the first viral PLA<sub>2</sub> domain to be crystallized. Expression of full length consecutive VP1 and VP2 structural proteins would encompass the future of this project. This would represent an important accomplishment in the field of structural virology. Contributions could be made towards the field of gene therapy as the molecular mechanism of parvovirus infection is revealed.

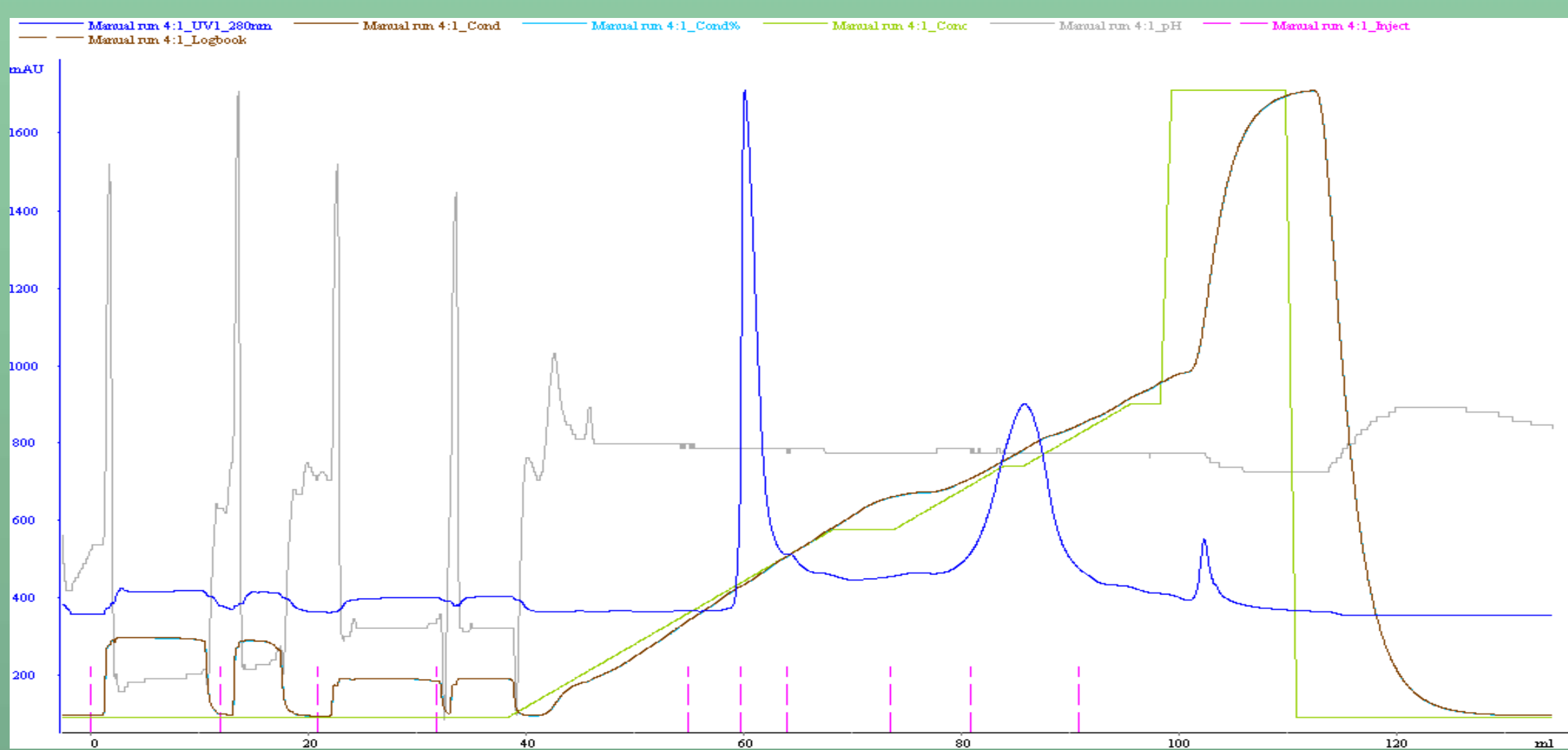


Figure A

The above figure is the HPLC chromatogram of a digested PLA<sub>2</sub> sample that has been injected four times into the ÄKTA explorer. A MonoQ ion exchange column binds the negatively charged protein with high affinity. Once the ionic strength is raised by adding a NaCl gradient, the fragments elute with high resolution. Blue peaks represent absorbance at 280nm indicating a high concentration of protein at that point. Fraction marks along the abscissa indicate volumes in which samples were collected for analysis. Fractions from 60-64ml were analyzed by mass spectroscopy in Figure B indicating a 14kD peak which is clearly the thioredoxin fragment. The second peak from 80-91ml was analyzed in Figure C showing two distinct bands at 14 and 26kD. This was a positive result demonstrating that the B19 fragment could be isolated and purified. However, there was still some contamination by the thioredoxin tag.

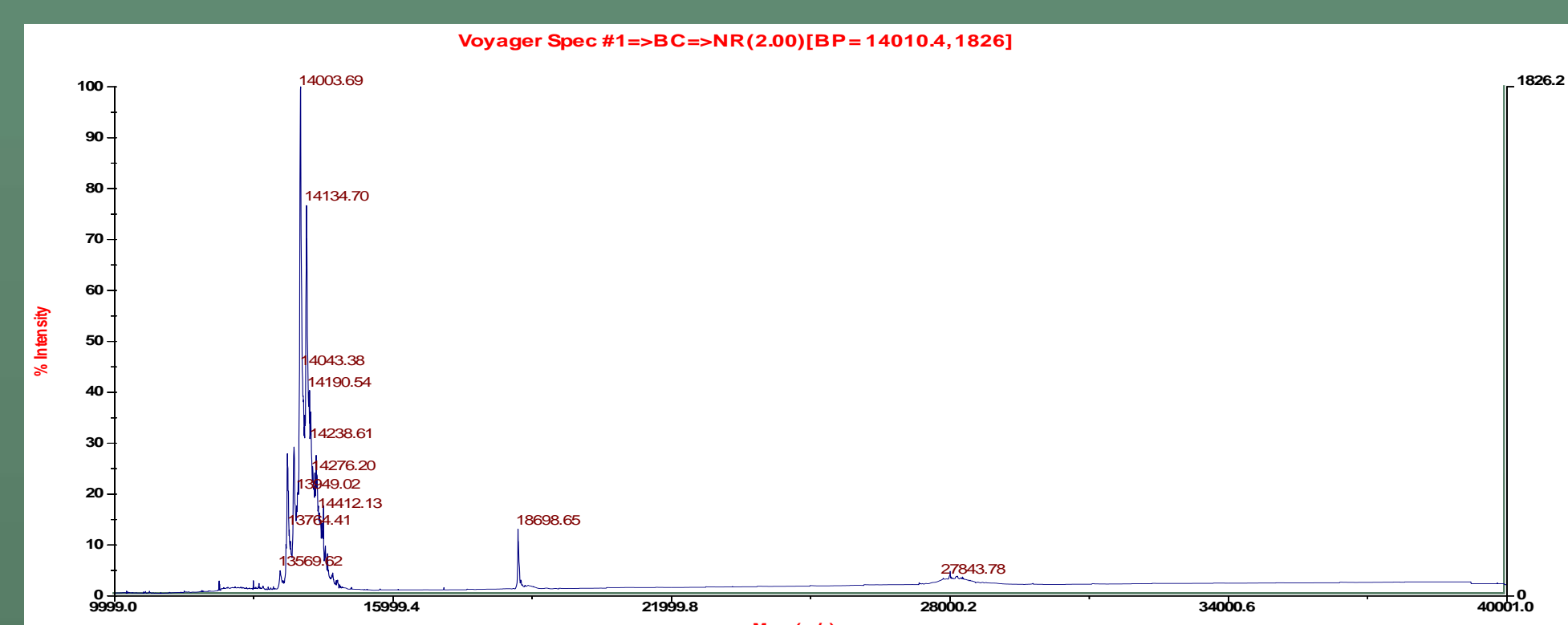


Figure B

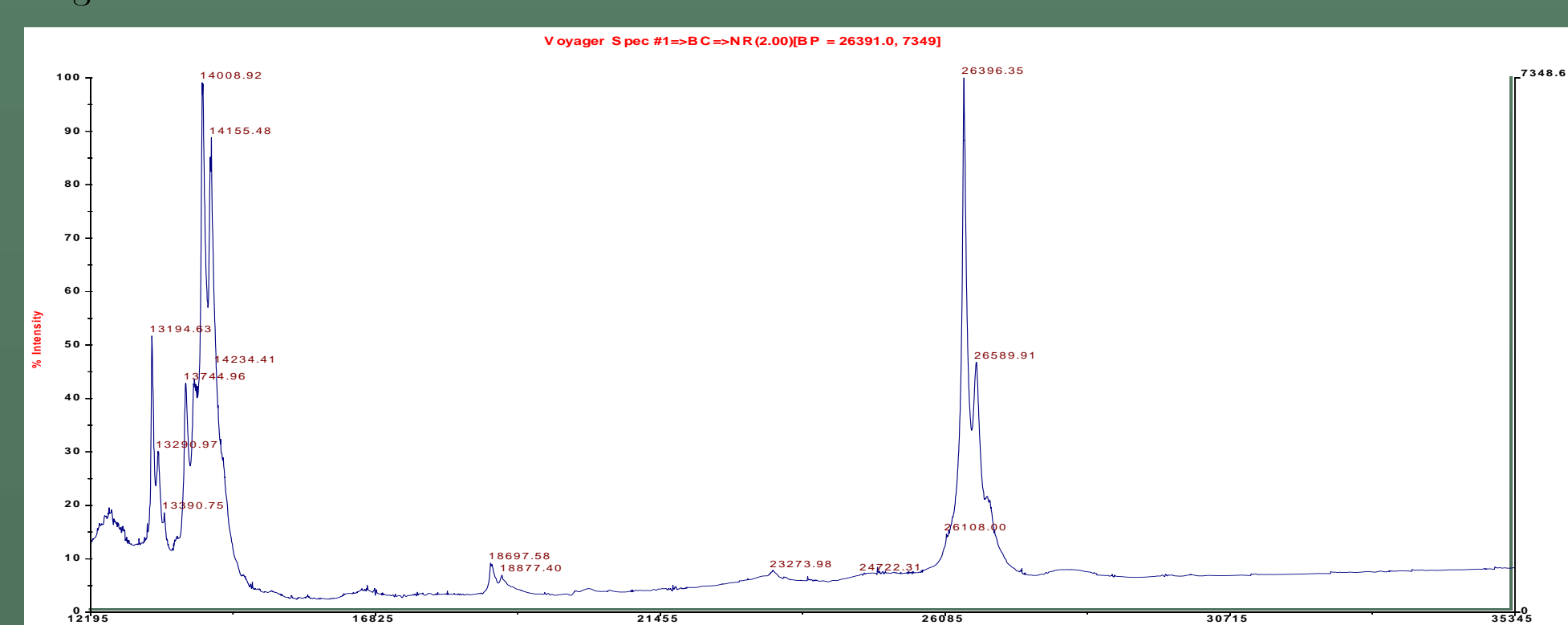


Figure C