Introduction:
- Bacteriophage (phage) genetic material is encapsulated in the capsid, or head of the phage. Due to the limited size of viral genomes the capsid consists of repeating units of certain proteins called capsid proteins. The icosahedral capsid consists of hundreds of copies of the major capsid protein (MCP) monomers.
- The MCP is an important biomarker for detection and analysis of phages. Further, the use of certain phages can be used to indirectly detect bacteria via phage amplification.
- Previous detection of capsid proteins by matrix assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) was limited by the ability to generate ions of the monomer that forms the phage capsid. The intact capsid, which consists of hundreds of copies of the MCP is well outside the operable mass range of MALDI-TOF MS.
- The method described here is based on the reduction of disulfide bonds responsible for cross linking between monomeric cystine residues, and allows for the disassembly of the capsid complex back into monomer units. The monomers can then be analyzed with MALDI-TOF MS.

Methodology:
- Previous work cites the use of acid pretreatment to liberate MCP monomers for detection/analysis. Samples were treated with formic and acetic acid at temperatures 30-80°C for up to two hours with no improvement in MCP detection.
- Because acid pretreatment only affects higher order structure caused by hydrogen bonding, it was hypothesized that other structural determinants such as disulfide bonds might serve as a suitable target for capsid disassembly.
- Samples of *Yersina pestis* bacteriophage Φ-A1122 (which is utilized for plague detection) were analyzed by combining 100μL of phage solution (1x10^9 pfu/ml) with 20μL of β-mercaptoethanol (βME) and allowed to react at room temperature for ten minutes prior to MALDI-TOF MS sample preparation.
- Mass spectrometric measurements were carried out with a 337 nm N₂ laser in linear mode using a PerSeptive Biosystems Voyager-DE STR+ MALDI-TOF-MS (Applied Biosystems, Inc. Framingham MA, USA). Spectra were collected with 25kV accelerating voltage, 75% grid voltage, and 100ns acceleration delay. Data was collected for triplicate spots with 250 shots per spectrum. Spectral data was exported from Data Explorer (Applied Biosystems) into Sigmaplot v11.0 for plotting and interpretation.

Conclusions:
- Previously less complex viruses were treated with acidic digests. This treatment was found to be ineffective for more complicated phages, as acidic treatment only targets structures formed from hydrogen bonding. More complicated phages require the reduction of disulfide linkages in addition to MALDI-TOF sample preparation, which is usually intrinsically acidic.
- Pretreatment with βME was found to reduce disulfide bonds resulting in disassembly of the MCP complex and thus liberating MCP monomers for detection in the operational mass range of MALDI-TOF MS.
- This method represents a reproducible and rapid pretreatment to readily detect phage MCP, which is an important biomarker in bacterial detection of phages in phage amplification based detection methods of interest.

References: