Silver nanoparticles (AgNPs) are well-recognized as antiviral agents but little is known about their mechanism of action. In this study, it was hypothesized that unfunctionalized, Creighton AgNPs of an average diameter of 11 nm will form covalent bonds with vaccinia virus (VV) mainly through the external, entry fusion complex (EFC) proteins. The EFC is housed on the external membrane of VV and contains 9-12 proteins having numerous cysteine groups, intramolecular disulfide bonds, aromatic moieties and myristic acids bound to the N-terminus of glycine residues. VV (1012 PFUs) was incubated at 37°C for one hour with Creighton AgNPs that were size selected (1-25 nm in diameter) and concentrated (1,000 ppm silver) using tangential flow ultrafiltration. After incubation, the sample was rinsed three times to remove any unbound AgNPs from the VV. The VV-AgNP sample was then deactivated with formaldehyde and fixed onto glass slides and stubs for surface-enhanced Raman spectroscopy (SERS) and scanning electron microscopy-energy dispersive X-Ray (SEM-EDX) analysis, respectively. SERS maps containing over 2,600 spectra were collected and processed using in-house written MatLab codes. Six endmember spectra were extracted from the hyperspectral data set using a multivariate statistical analysis method, namely vector component analysis (VCA). The SERS analysis of the six endmember spectra indicated an interaction trend similar to that reported in literature by other SERS studies on proteins exposed to AgNPs: carboxylic groups > peptide bond interactions (amide peaks) > aromatic AAs > thiol groups > small side chains. Additionally, the SEM electron backscatter images and the EDX spectrum of the VV-AgNP sample revealed the presence of silver and further supported the direct interaction between AgNPs and VV. These interactions confirmed the proposed hypothesis and suggested that the covalent bonding interactions might disrupt the VV ability to complete the entry/fusion steps of the viral replication cycle.