

Abstract

Due to the significant impact viruses pose to public health, the economy, and for their potential to be used as biological weapons, novel non-traditional methods are needed to characterize emerging viruses. As virus particles bud from an infected cell, they contain not only virus-specific protein and nucleic acid, but also incorporate host proteins. While this phenomenon has been observed for a handful of viruses, it remains unclear if host proteins are specifically packaged and if they are critical to the viral life cycle. **In this study, the protein content of purified virions from the *Alphaviridae* and *Arenaviridae* families will be analyzed by liquid chromatography mass spectrometry to determine if host proteins are specifically packaged by these viruses and if these host proteins are critical for the viral life cycle.** The purified virions will first be propagated and purified from the same host cells, thus giving a common background and identical set of host proteins for bioinformatic comparison both within and between viral families. To determine if observed commonalities are host cell specific, the same study will be repeated in a new host cell line and then compared in years 2 and 3. If virus particles package specific host proteins and there are identifiable patterns of host proteins within or between viral families, then **these molecular signatures could provide a basis by which new strategies could be developed for viral origin, characterization, detection, and treatment.**

Sindbis Virus Virion Purification

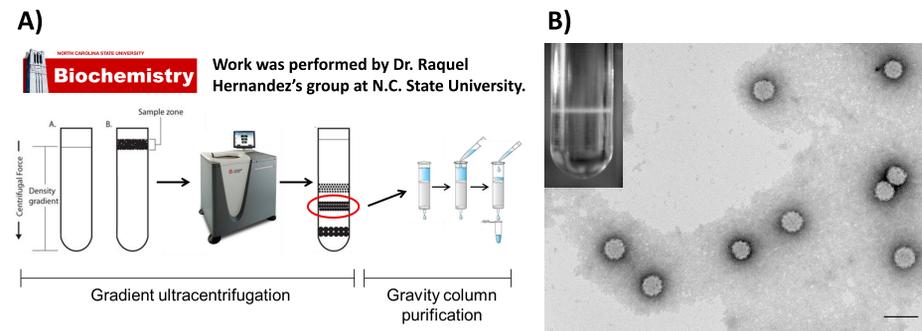


Figure 2. Sindbis virus (SINV) virion purification strategy and production timeline. A) A two step purification strategy was utilized; the virus preparations were first spun over density gradients using an ultracentrifuge then eluted using gravity columns. Negative controls were processed in parallel with the virus preparations. B) Electron micrographs of the purified virions to determined that the two step purification strategy provided pure preparations of virions for downstream mass spectrometry analysis.

Host Protein Functional Analysis

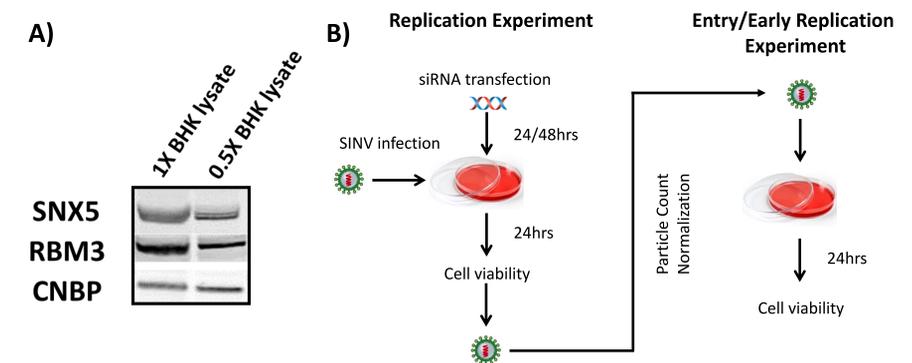


Figure 4. Functional analysis workflow for identified host proteins. A) Sourced rabbit polyclonal antibodies raised against human derived proteins for SNX5, RBM3, and CNBP correctly identified these host proteins in hamster backgrounds. This demonstrates our ability to detect knockdown of these proteins and thus assess their functional relevance to the SINV life cycle (Figure 1). B) Workflow for the functional analysis of host proteins using siRNA. The initial knockdown of host proteins will determine if they have a functional role in the initial infection life cycle. Virus particles produced from these cells containing the knock down will be collected, normalized, and then used to infect WT host cells. This strategy will allow for us to determine if there is a functional role for these host proteins for initial as well as progeny virus infection.

Viral Life Cycle and Host Proteins

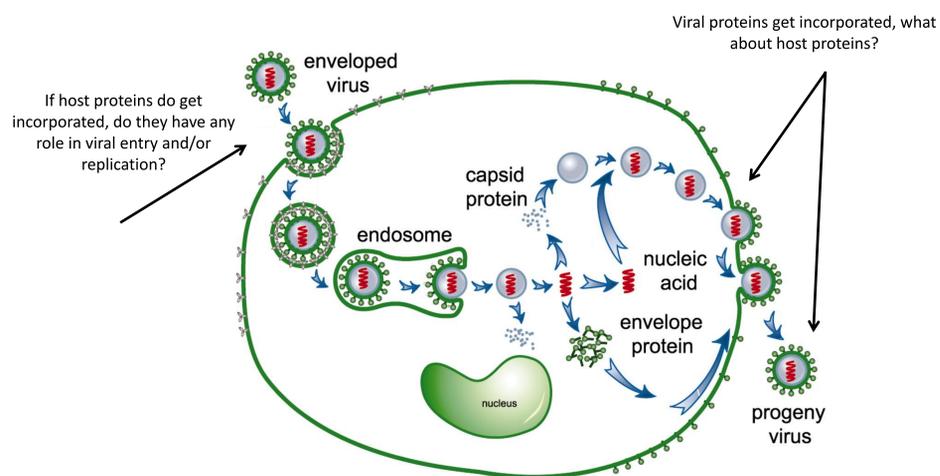


Figure 1. Enveloped virus life cycle and the role for host proteins. Enveloped viruses enter host cells via fusion at the plasma membrane or endocytosis and then ph-mediated fusion within the endosome. This membrane fusion then transports viral proteins and nucleic acids into the host cell cytoplasm where viral replication occurs. Viral structural proteins are produced with viral genomes, and these are packaged into progeny virions and released from the host cell. To determine if proteins from the host cell are being incorporated into virus particles, the following tasks will be performed: In FY15, this basic research study aims to 1) grow and purify viral particles from at least two different members of both the Alphavirus and Arenavirus families, 2) extract and digest protein from purified virions, 3) analyze tryptic peptides by liquid chromatography mass spectrometry (LC-MS), 4) process spectra data to determine the host and viral protein content, and qualitatively and quantitatively compare the proteomes of each virus. In year two of the proposed project, additional work will be carried out 5) to see how changing the host will affect the presence or absence of host proteins and 6) to determine if these host proteins, when knocked out, affect the virus's life cycle.

MS Analysis of Virus Particles

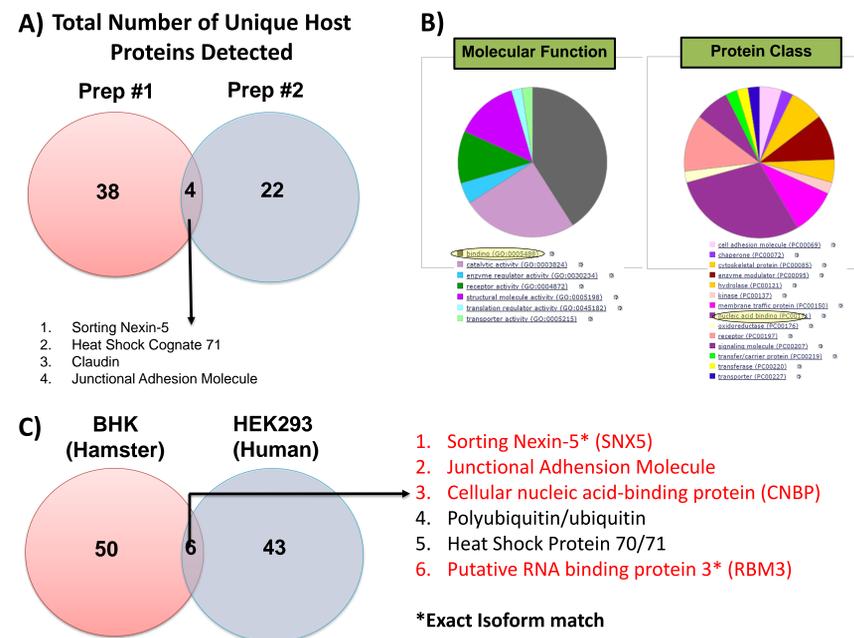


Figure 3. LC-MS-MS Host Protein Identification from SINV virions . A) Multiple preparations of SINV virions produced from BHK (baby hamster kidney) cells led to a significant amount of host proteins identified. When comparing the preparations to each other, we identified four proteins that had multiple signatures detected in both preparations. These signatures identify proteins in higher concentrations within these virus particles. B) The functional classification of all the host proteins identified within virions from both hamster and human backgrounds were determined using PANTHER. Highlighted are the function and classification of potential target proteins for functional characterization. C) In addition to comparing preparations within hosts, we also compared the host proteins identified between hosts. Using this strategy we identified 6 protein signatures that were conserved between both host backgrounds. This list serves as our down-select for functional analysis using siRNA (Figure 4).

Conclusions and FY16 Tasks

The first year of this ILIR-funded project has yielded host protein identification in three host backgrounds for Sindbis virus, the prototypical alphavirus. This data has allowed for us to:

- Characterize novel virion proteomes (virus and host proteins) from potential biological threat agents
 - Drive hypotheses for the development of host targets for antiviral activity and/or detection
 - Help develop the understanding of basic virus biology
 - Create a functional picture of unannotated mosquito host proteins when compared to the host proteins identified from hamster and human backgrounds
- The work presented here will continue to drive our research into year 2 of the ILIR project, with the following products resulting from work in FY16/17:

Deliverables:

- Virion proteome for these viruses is unknown, so even the characterization is novel (presentations/publications)
- Curated database for host proteins within virion particles
- Potential host-targeted therapeutic targets
- Peer-reviewed publications

Transitions:

- Virion proteome characterization can aid in forensic applications and assay development (virion proteome database)
- Potential therapeutic targets have medical applications