Encapsulation of Proteins Inside The HK97 Virus Like Particle

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Introduction

Proteins are important biologically active molecules that are utilized for many important applications ranging from therapeutics in human disease to protein catalysts called enzymes utilized for industrial processes. However, proteins are often found to be unstable and prone to inactivation, denaturation, and degradation, sometimes limiting their use in some applications. Developing new strategies to improve the stability and protecting them from inactivation is attractive in broadening their use and effectiveness for applications. One strategy is to encapsulate the proteins inside stable nanocontainers that protect and prevent their inactivation. Here we describe the investigation of the encapsulation of proteins inside of a protein cage structure derived from a virus, termed a virus like particle (VLP). The VLP platform examined is derived from the bacteriophage Hong Kong 97 (HK97) (**Figure 1A**), a virus that infects *Escherichia coli.* The HK97 virus is unique in that its capsid head forms a catenane structure, where all protein subunits are chemically crosslinked to form the equivalent of molecular chain mail, that is nearly indestructible. The HK97 VLP self assembles from 420 copies of the 42 kDa major coat protein, GP5, and it has been shown that it can be matured into the unique catenane. For maturation HK97 employs a maturation protease (gp4), that is encapsulated on the interior of the immature capsid during assembly. Proteolytic cleavage of the GP5 protein by GP4 leads to maturation of the phage or VLP into the mature catenane structure with high stability and a molecular diameter of 66 nm. The research presented here looks at exploiting the HK97 VLP as a scaffold for the encapsulation of proteins through an efficient *in vivo* strategy.

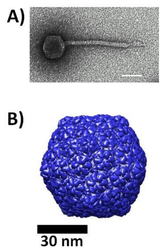


Figure 1. The bacteriophage HK97. TEM image (A) and crystal structure (B) of the HK97 bacteriophage

HK97 Capsid Assembly Pathway and Gene Organization

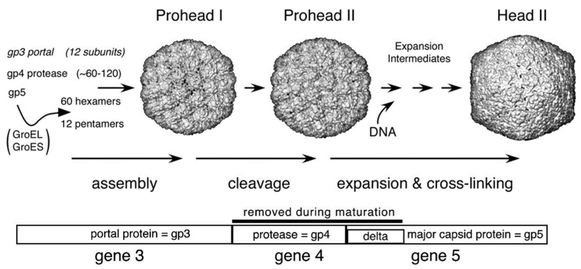


Figure 2. HK97 assembly pathway. A schematic for HK97 assembly, showing how the three HK97 capsids transform from Prohead I all the way to Prohead III. Map of the three contiguous HK97 capsid HK97 genes.

- Viral assembly requires the expression genes encoding the portal protein (GP3), HK97 protease (GP4), and coat protein (GP5) that are arranged in a linear manner on the viral operon.
- The GP4 protein becomes encapsulated and catalytically cleaves GP5 protein subunits allowing transition to the mature head form of the catenane viral capsid.

HK97 as a Container for Encapsulating and Stabilizing Enzymes

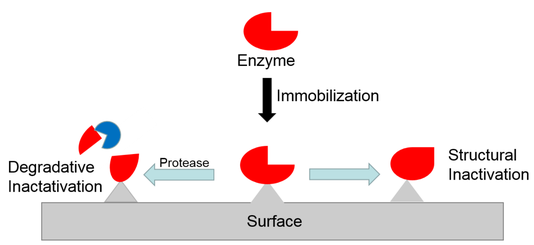


Figure 3. Enzyme stabilization is needed for applications requiring enzyme immobilization.

- The HK 97 VLP is Very Stable (withstands 80 °C heating).
- Large hollow interior for protein encapsulation.
- 2 nm sized pores in the cage allow small molecules to enter and exit.
- High yield (~50 mg protein/L of media) of VLPs by expression of GP5 in *E. coli.*

Chemically Programmed Encapsulation of Proteins Inside HK97

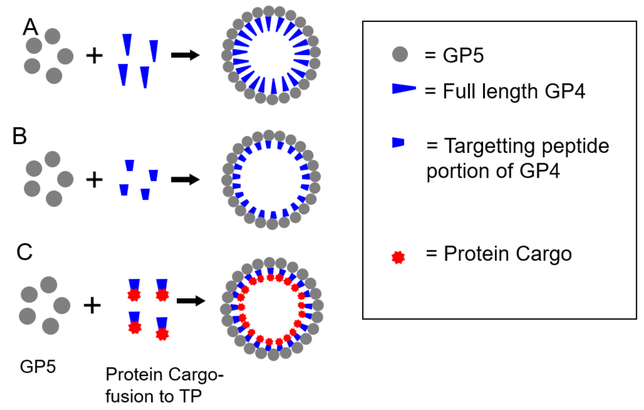


Figure 4. Strategy for encapsulation of foreign protein cargoes inside the HK97 VLP.

- A targeting peptide sequence (TP) from GP4 directs encapsulation of GP4 inside the HK97 VLP.
- Attachment of the TP sequence to a cargo protein is expected to lead to encapsulation.

Approaches For HK97 Encapsulation of Proteins *In vivo*

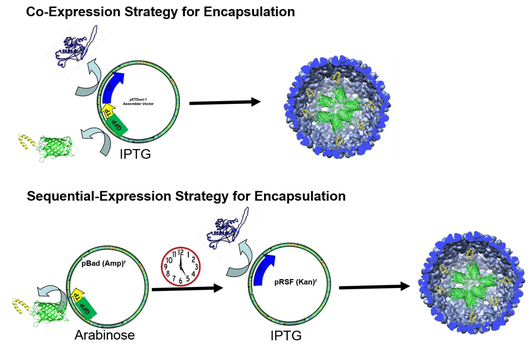


Figure 5. Strategies for the expression and encapsulation of GFP inside the HK97 VLP.

- GFP serves as a visual tag for encapsulation and quantitation of encapsulation.
- GFP was inserted into plasmids (NcoI/BamHI restriction sites) with TP (BamHI/SacI).
- Examine co-expression vs. sequential to determine the best incorporation of GFP-TP.

Expression and Purification of HK97 Encapsulating GFP

Figure 6. Pellets of Sequential (left) and Coexpressed (right) VLP.

- Sequential expression was performed by expressing GFP-TP overnight by induction with Arabinose, then induction of GP5 by IPTG for 6 hours.
- Co-expression was carried out by induction overnight with IPTG.
- Cells were pelleted, resuspended in PBS (phosphate buffered saline), lysed by sonication, insoluble cell matter removed by centrifugation, and HK97 VLPs purified by ultracentifugation of cell supernatant through a sucrose cushion.
- HK97 VLPs were subsequently resuspended overnight in PBS and subsequently purified by size exclusion chromatography.

Characterization of HK97-GFP VLPs by Ultracentrifugation

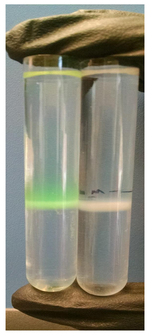
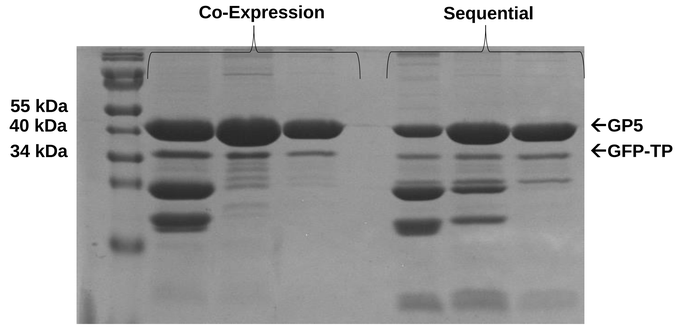


Figure 7. Cesium Chloride Analysis.
GFP left (Green) and VLP on the right.

- Ultracentrifugation through cesium chloride showed a co-migration of GFP color with the HK97 VLP.
- Comparison of HK97-GFP VLPs (green) against wild type HK97 (white) showed same migration pattern.

SDS-PAGE Analysis and Dynamic Light Scattering (DLS)



Sample	Average & S.D of HK97 VLP Size (nm)	Average & S.D of Number of GFPs/VLP
Co-Expression	53.3 ± 1.24	88.0 ± 39.7
Sequential Expression	59.0 ± 1.63	96.0 ± 44.5

Figure 8. SDS Page gel analysis of HK97 VLPs. Top) SDS-PAGE gel. Lane 1: Molecular weight standard; Lane 2: Co-ex 1; Lane 3: Co-ex 2; Lane 4: Co-ex 3; Lane 6: Seq 1; Lane 7: Seq 2; Lane 8: Seq 3. Expect 42 kDa for GP5 coat protein. **Bottom)** Table showing the quantitative results for DLS and SDS-PAGE for three replicates.

- Ratio of band intensity from the GP5 band (42 kDa) and GFP-TP (38 kDa) was analyzed by densitometry.
- Ratio of GFP-TP to CP indicates better packaging by sequential method.

Summary and Future Directions

- Attachment of the TP to GFP enabled encapsulation of a foreign protein inside the HK97 VLP
- Preliminary studies suggest co-expression is better than sequential in the number of GFPs encapsulated per HK97 VLP
- GFP over-expresses and other proteins that don't express as well might work better with the sequential strategy in the future
- Future research will explore the encapsulation of enzymes, such as CelB, SULT1a1 & Nit1, and their ability to be stabilized by encapsulation inside the HK97 VLP

Acknowledgements

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