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Investigating Programming of a Virus Like Particle for Cell Specific Chemotherapy

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Introduction

Cell specific drug delivery is a major challenge in chemotherapeutic strategies targeting, particularly in cancer strategies. Side effects for non-specific uptake of chemotherapeutic drugs by normal healthy cells include hair loss, vomiting, nausea, and other negative symptoms. The use of nanoparticles such as those derived from protein cage structures present potentially useful agents for drug delivery by encapsulation of drugs on the interior of the protein cage nanoparticle. However, while strategies for encapsulation of drugs are plentiful, methods for programming site/cell specific delivery of nanoparticles are needed. One potential protein cage platform for drug delivery is the virus-like particle (VLP) derived from the bacteriophage Hong Kong 97 (HK97). The HK97 VLP self assembles from 420 copies of the GP5 coat protein to form a hollow icosahedral 56nm structure. HK97 is an interesting protein because it has been observed to require cell targeting moieties to be added to the exterior for entry into cells. The research presented here looks to examine a proof of concept strategy for localizing HK97 VLPs based on chemo-sensitive programming of the exterior. The approach looks at exploiting the property of cancer cells to upregulate metalloproteases in the cancer microenvironment. We hypothesize that peptides can be added to the outside of the HK97 VLP that program the localization and deposition of HK97 VLPs loaded with drugs when proteases are present. To evaluate this hypothesis we examine the genetic attachment of a model peptide containing poly-phenylalanine flanked by an aspartic acid and a solubilizing poly-lysine peptide sequence to the GP5 protein C-terminus. Treatment with N-aspartate protease, a "model" protease, is expected to lead to VLP self-assembly and aggregation in support of our proposed hypothesis.

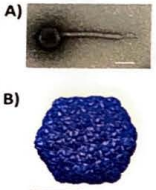


Figure 1. The bacteriophage HK97. A) TEM image of the HK97 bacteriophage. B) HK97 Head model derived from the x-ray crystallography structure.

Properties of the HK97 VLP Ideal for Drug Delivery

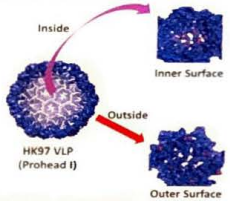
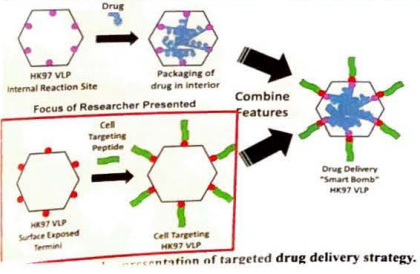


Figure 2. Internal and external structural characteristics of HK97 VLP. Inner surface of the HK97 has exposed amino acid esidues (magenta and orange). Exterior surface has the C-erminus of GP5 (red) exposed.

- Large hollow interior for drug loading.
- Drugs can diffusion through 2 nm sized pores in the cage.
- Exposed C-terminus as an attachment site on exterior (**Figure 2**).
- Highly stable for delivery *in vivo*.
- Ideal nanoparticle size for accumulation in cancerous tissue (EPR effect).

Manipulating HK97 VLP For Encapsulation and Self Assembly



...esentation of targeted drug delivery strategy.

- Engineer the interior surface for allowing localization of therapeutics.
- Engineer exterior surface for attachment of cell targeting moieties.
- Combining internal drug packaging with external modification for cell targeting will produce "smart bomb" drug delivery nanoparticles.

Targeting Metalloproteases

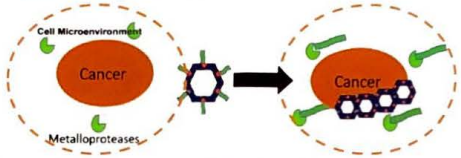
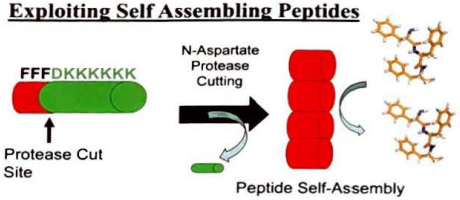


Figure 4. Schematic of Metalloprotease Activity with VLP in Cancer Microenvironment

- Cancer microenvironments are known to contain proteases, which are proteins that cut/digest other proteins at specific amino acids.
- The VLP exterior can be programmed with peptides that are modified/removed by metalloproteases that are in the microenvironment of the cancer, but are not found elsewhere.
- Designing the peptides to cause self-assembly upon digestion/cutting by the protease is expected to localize the drug delivery VLP

Exploiting Self Assembling Peptides



- The de novo design of peptides that self-assemble through non-covalent interactions into higher order structures is well studied.

Figure 5. Representation of Process of Cutting and Assembling the HK97 Peptide by Pi-Stacking

- Peptides comprised of 2 or 3 consecutive phenylalanine amino acids (F) are known to spontaneously form large nanostructures (tubes or spheres) through Pi-stacking interactions between F residues.
- Design of a peptide containing F residues that prevents Pi-stacking until modified by a protease is a potential strategy for chemically programming cell targeting by exploiting the self-assembly properties of the poly-F peptide
- Amino acids that repel and maintain solubility, such as lysine (K), are expected to prevent assembly of poly-F peptides

Incorporating Site for Targeting Moieties

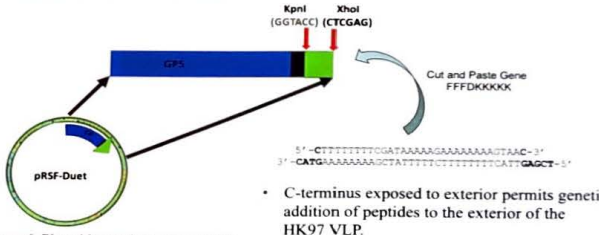


Figure 6. Plasmid containing HK97 GP5 gene designed for modular incorporation of peptide genes. Design features include a modular poly-glycine spacer (black) between the GP5 coat protein (blue) and peptide insert (green) that allows spatial flexibility and separation upon gene expression.

- C-terminus exposed to exterior permits genetic addition of peptides to the exterior of the HK97 VLP.
- Plasmid was constructed enabling peptide sequences to be added in a modular fashion using KpnI and XhoI restriction enzymes (**Figure 6**).
- Peptides appended could be used directly for cell targeting or as molecular handles to attach targeting moieties.

Colony PCR Screening of Gene Insertion

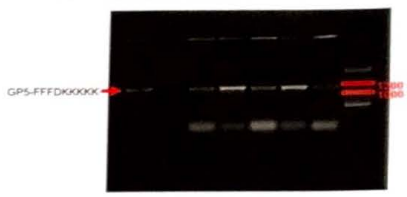


Figure 7. Gel Results from Colony PCR of Colonies 1-6 and Standard Ladder

- DNA was extracted from E. Coli colonies
- The DNA underwent Polymerase Chain Reaction (PCR) and then was run on a gel
- The gel is visualized in **Figure 7** and includes a standard protein ladder
- Based on the GP5 coat protein, colonies that had the gene inserted in the plasmid would have hits at approximately 1200 base pairs
- The colonies with the boldest bands at approximately 1200 BP indicated by the red arrow were selected for further testing.

Sequencing of Gene Inserts

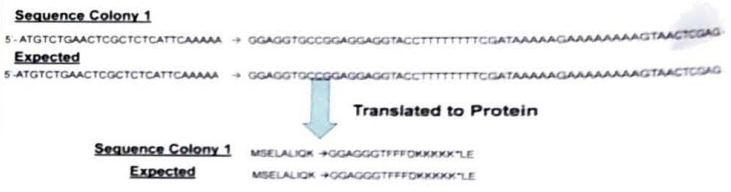


Figure 8. Sequencing and Protein Translation Results of Colony One DNA

- DNA was extracted from the E. coli that received "hits" by PCR for the inserted gene
- Sequencing was performed by Eurofins Operon sequencing service
- All hits showed correct sequence expected for attachment the FFFDKKKK gene at the C-terminus of GP5 gene

Summary and Future Directions

- Gene encoding FFFDKKKK has been successfully incorporated into the expression vector for GP5
- Trials are underway to evaluate the expression of the modified HK97 VLP Gene encoding FFFDKKKKK.
- Modified HK97 VLPs will be characterized to determine their ability to self-assemble after N-Aspartate Protease treatment
- Designs of peptides that will allow targeting of metalloproteases in cancer cell microenvironments are being examined in the case that our proof of concept studies are successful.

Acknowledgements

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