

# Targeted *In vitro* Drug Delivery to Human Hepatocarcinoma via a Virus-like Particle Carrier

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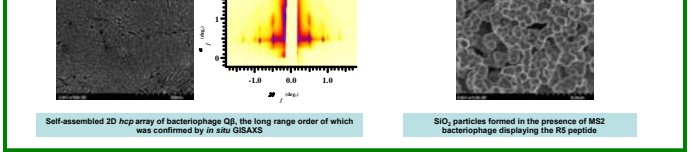


## INTRODUCTION

Virus-like particles (VLPs) possess characteristics that make them well-suited for a variety of biological and materials science applications:

- VLPs are highly monodisperse, enabling their self-assembly into well-ordered 2D structures by simple evaporation-driven techniques.
- Phage capsids can be engineered to express non-native peptides with known affinities. These peptides can: (1) nucleate gold, zinc sulfide, etc. from precursor salt solutions (e.g. p89, which binds gold); (2) condense silica from silicic acid at neutral pH (e.g. R5 repeat unit from silaffins); (3) detect the presence of surface antigens expressed by pathogens (e.g. LPS-reactive peptides); (4) produce monoclonal antibodies against a given pathogen (e.g. anthrax protective antigen); and (5) target drug carriers to a specific cell type (e.g. SP94, which targets hepatocarcinoma).

VLPs can be used as a platform for phage display, a combinatorial-based technique that enables selection of peptides that bind to a specific material (organic, inorganic, biological) from a complex random peptide library.



## TARGETED VLP-BASED DRUG DELIVERY

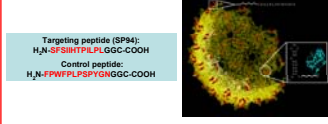
Virus-like particles of MS2 and Q $\beta$  bacteriophages are well-suited for use as targeted drug delivery carriers:

- MS2 and Q $\beta$  are cheaply produced and purified in large quantities
- Their 28-nm capsids self-assemble from 180 copies of coat protein
- Their capsids are tolerant of high density peptide display
- Their capsids self-assemble around RNA genome and will encapsidate RNA-cargo conjugates
- Their capsids are stable under physiological conditions and are biocompatible

MS2 and Q $\beta$  VLPs can be used as targeted drug delivery systems by internal and external modification of their capsids:

### External Capsid Modification with Targeting Peptides

- Link surface Lys residues in coat protein to peptides with a C-terminal cysteine residue via a heterobifunctional crosslinker (SMPH)

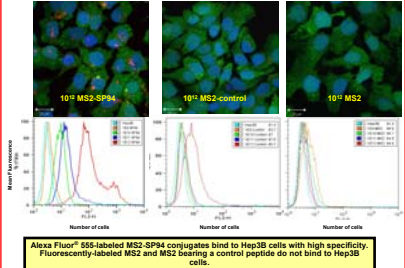


### Internal Capsid Modification with Various Cargo

- Link various cargo (drugs, siRNA, QDs, Au NPs, Fe<sub>3</sub>O<sub>4</sub> NPs, etc.) to thiolated RNA

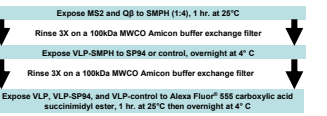
Lo, A., Lin, C-T and Wu, H-C. (2008). Hepatocellular carcinoma cell-specific peptide ligand for targeted drug delivery. *Mol. Cancer Therapy* 7 (3): 570-586.

## TARGETED MS2 BINDS TO HEPATOCARCINOMA

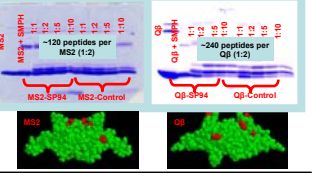


## PEPTIDE CONJUGATION

Higher peptide conjugation efficiencies can be achieved with Q $\beta$  due to the increased availability of surface lysine residues:

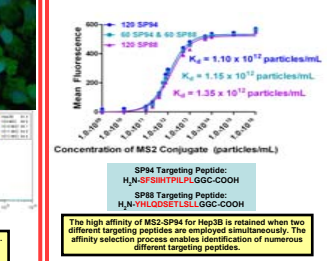


Conjugate	Dye Molecules per Capsid
MS2-dye	168
MS2-SP94-dye	149
MS2-control-dye	147
Q $\beta$ -dye	182
Q $\beta$ -SP94-dye	61
Q $\beta$ -control-dye	49



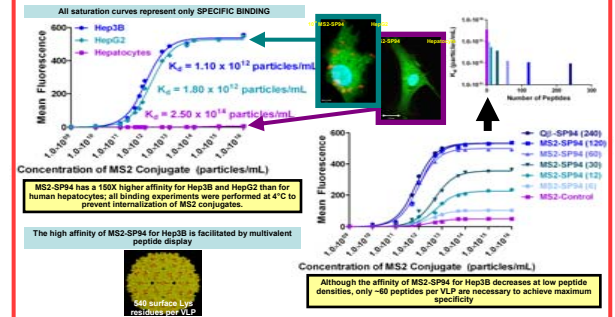
Although higher conjugation efficiencies can be achieved with Q $\beta$ , we have more thoroughly studied MS2 (e.g. tolerance to peptide display).

## USE OF MULTIPLE TARGETING PEPTIDES MAY HELP MITIGATE AN IMMUNE RESPONSE

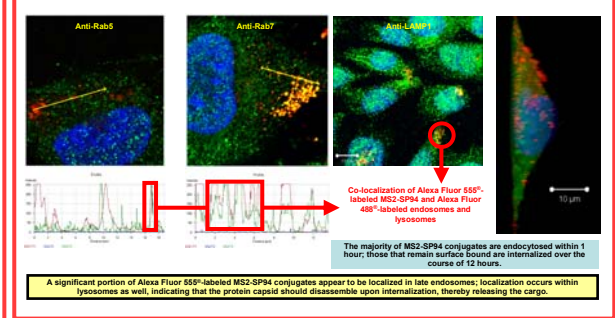


## Surface Binding and Internalization of MS2-SP94 Conjugates

### MS2-SP94 HAS A 150X HIGHER AFFINITY FOR HEP3B THAN FOR HEPATOCYTES

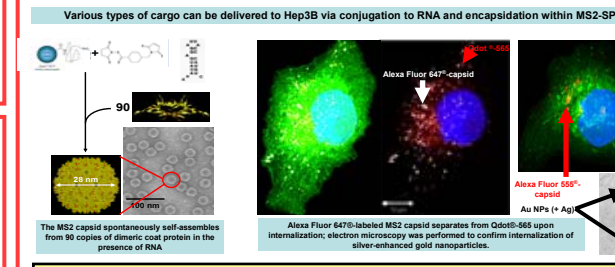


### MS2-SP94 CONJUGATES ARE ENDOCYTOSED BY HEP3B



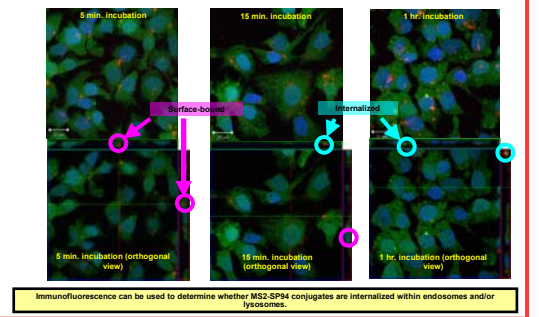
## Future Directions

### DELIVERY OF QDs AND NPs

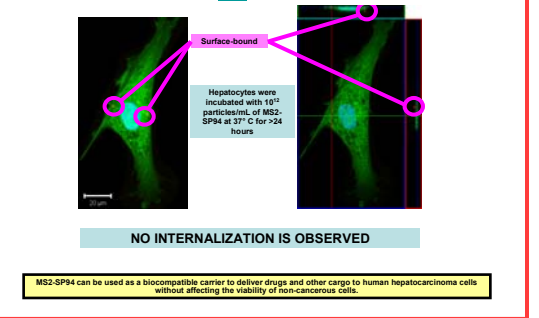


The SP94 peptide mediates HIGHLY specific binding to and internalization of virus-like particle (VLP) drug carriers within human hepatocarcinoma Hep3B, while minimizing interaction with human hepatocytes. We are currently investigating the delivery of multiple types of cargo: Fe<sub>3</sub>O<sub>4</sub> particles, siRNA that silences cyclin A expression, cholera toxin A chain, and ricin toxin A chain

### INTERNALIZATION OF MS2-SP94 OCCURS PRIMARILY OVER ONE HOUR



### MS2-SP94 CONJUGATES ARE NOT INTERNALIZED BY HEPATOCYTES



### DELIVERY OF DOXORUBICIN

