UTILIZATION OF FLOCKHOUSE VIRUS-LIKE PARTICLES FOR THERAPEUTIC PURPOSES

MARYAM KHURSHEED



KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

INDIAN INSTITUTE OF TECHNOLOGY DELHI

JUNE 2026

© Indian Institute of Technology Delhi (IITD), New Delhi, 2026

UTILIZATION OF FLOCK HOUSE VIRUS-LIKE PARTICLES FOR THERAPEUTIC PURPOSES

by

MARYAM KHURSHEED

Kusuma School of Biological Sciences

Submitted

In fulfilment of the requirements of the degree of

Doctor of Philosophy

to the



Indian Institute of Technology Delhi, India

JUNE 2026

“To Amma and Abba — the reason behind every achievement, the strength behind every struggle.”



"Taught man that which he knew not"

CERTIFICATE

This is to certify that the thesis entitled **"Utilization of Flock House Virus Like Particles (VLPs) for Therapeutic Purposes"** being submitted by **Ms. Maryam Khursheed** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi** for the award of the degree of **"Doctor of Philosophy"** is a record of the bonafide research work carried out by her, prepared under my supervision, in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research report and the results present in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Date:

Place:

Prof. Manidipa Banerjee
Professor
Kusuma School of Biological Sciences
Indian Institute of Technology, Delhi
Hauz Khas, New Delhi- 110016 India

DECLARATION

I hereby declare that:

1. The work presented in this thesis is original and has been carried out by me as a student of IIT Delhi.

2. To the best of my knowledge, the work presented in this thesis has not been submitted, either in whole or in part, to any other institution for the award of any degree or diploma.

3. I have adhered to the guidelines prescribed by the Institute for thesis preparation.

4. I have complied with the provisions of the Institute's Honor Code and have neither engaged in nor tolerated any form of academic dishonesty, including plagiarism, intentional data fabrication, data falsification, data misrepresentation, or unauthorized collaboration beyond that permitted by the Student Research Committee, as defined and approved by the Institute.

5. I take full responsibility for all data presented in this thesis and confirm that no data has been collected without appropriate permission. Where required, ethical approval has been obtained for the research, and all relevant approvals and documentation have been included as part of the thesis submission.

6. All sources of information, including data, ideas, and text, have been duly acknowledged and appropriately cited in the thesis.

7. The use of artificial intelligence (AI) tools (if any), including large language models, has been appropriately disclosed. All analysis interpretations, and conclusions presented in this thesis are my own. Alternatively, I declare that no AI tools, including large language models, have been used in the preparation of this thesis.

8. I understand that any violation of the above provisions may lead to appropriate action as per Institute rules, including withholding of degree approval or revocation of the degree, as applicable under Institute regulations.

Date: 15th June 2026

Place: New Delhi

(Signature of the Student)

Name of the Student: Maryam Khursheed

Entry Number.: 2018BLZ8465

Acknowledgements

Alhamdulillah — all praise belongs to Allah, the Most Merciful, the Most Gracious, for granting me the strength, patience, and resilience to see this journey through to its end. This PhD has not been a solitary pursuit of knowledge but a journey of faith, resilience, and the quiet, unwavering support of those who walked beside me in seen and unseen ways.

Beginning with my supervisor, ***Prof. Manidipa Banerjee****. As a woman scientist, her ability to handle everything with so much composure and grace, juggling academic and personal obligations along with all the research enthusiasm, and never letting down on any front, is inspirational. She has taught me things that go much beyond lab and experiments. It has been an honour to work under her mentorship, and I will always look up to her as a source of inspiration in both academic and personal life.*

My Doctoral committee members, Dr. Tapan. K. Chaudhuri, Dr. Archana Chugh, and Dr. Subhash. K. Yadav for their valuable time, thoughtful critique, and constructive feedback, which helped strengthen this work and enriched my academic perspectives. Our collaborators, Dr. Avinash Bajaj and Dr. Poonam Yadav, for their valuable suggestions and resources throughout the project.

My lab seniors, especially Dr. Sukanya Ghosh, who held my hand from the very first day in the lab, guided and supported me patiently, taught me everything from the most basic experiments to the complex, intricate protocols. Dr. Ramesh Kumar, whose kindness and generosity in answering my endless queries, explaining concepts, no matter how trivial, have nurtured my confidence as a researcher. My other lab mates, past and present- Dr. Debajit Dey, Dr. Subhomoi Borkotoky, Dr Akanksha Saini, Dr Pankhuri Narula, Manthan Raj, Pushkar Tatiya, Kirti Suhag, Paulomi Goswami, Mrittika Dasgupta, Rupam Khatua, Himanshu, and Komal Arora, for being the most amazing set of people to work with.

My beautiful circle of friends, Pragati, Aditi, Vikas, Ram, Prasanjeet, and Milan, you guys have been a steady source of strength through every season of this journey, and my biggest cheerleaders in times of success. Thank you for always checking in, for making sure I was doing fine even when I withdrew into work or worry, and for reminding me that life exists outside of research.

we've beautifully grown to be part of each other's lives, sharing celebrations, struggles, and everything in between. This bond we've built, of endless conversations and mutual care, is one I know will remain for a lifetime.

To some more amazing souls I met during this journey, Pradeepti, Nadira, Khadija, Uzma, Devanshi,, from troubleshooting to impromptu tea breaks that turned into heart-to-heart conversations, your friendship has meant more than you know. Thank you

To my Amma, ***Mrs. Shabnam Khursheed,*** *Abba,* ***Mr Khursheed Alam*** *and Bhaiyya* ***Dr. Mohd Talha Khursheed,*** *you are the heart of everything I am and all that I will ever be. Every step of this journey has carried your love, prayers, and sacrifices within it. Amma You've always believed in me, even in the moments I couldn't believe in myself. Your words, your duas, and your unconditional love have been my safe place through every storm. Abba you taught me perseverance, and the value of hard work. Every late night, every challenge, every small success I carried both of you with me through it all. This PhD is not just my achievement; it is yours, born of your unwavering belief and the countless prayers you made for me.*

To my husband, ***Dr. Monish Ahmad****, you have been my anchor throughout this journey. Thank you for your unconditional love, endless patience, and for carrying my burdens as your own. Your support and faith in me gave me strength when I had none. This achievement belongs as much to you as it does to me.*

For everything I have achieved, I owe a part of it to each of you. Thank you for helping me become who I was meant to be.

Maryam Khursheed

ABSTRACT

Targeted drug delivery for the treatment of diseases like cancer is a major challenge because of the side effects associated with the available treatment options. A competent delivery system is needed that can address the challenges associated with the delivery of molecules to the target sites. The structural proteins of many viruses can self-assemble spontaneously into virus-like particles (VLPs) under appropriate conditions, which makes them potentially effective nanocarriers for drug encapsulation and delivery.

We have exploited the inherent advantages of an insect nodavirus, Flock House Virus (FHV), to engineer a targeted nano-vehicle for site-specific peptide functionalization, hydrophobic drug encapsulation, and targeted delivery in melanoma and colorectal cancer models, establishing a modular framework for receptor-directed nanotherapeutics.

Since FHV lacks mammalian cell tropism, making use of thiol-maleamide-based conjugation, we have devised a method to attach targeting peptides to FHV virus-like particles (VLPs) via polyethene glycol based crosslinker. The attachment of the peptides transformed the VLPs' lack of tropism and enabled them to selectively bind with the receptors overexpressed on cancer cells. The optimised conjugation protocol ensured the particles' symmetry and overall stability. Further, the natural affinity of FHV for hydrophobic molecules has been utilized for encapsulating hydrophobic chemotherapeutic drugs, like doxorubicin, by transient destabilization and re-stabilization of the capsid via modulation of Ca2+ concentration. The resulting modified VLPs exhibited high drug-loading capacity and pH-responsive release behaviour, with minimal leakage at physiological pH and accelerated release under acidic endosomal conditions.

These modified drug encapsulated VLPs have demonstrated controlled and sustained cytotoxic effect primarily through apoptosis in comparison to the rapid and non-controlled cell death induced by free drug. The drug-encapsulated VLPs display high anticancer efficacy in the murine colorectal model, leading to tumour suppression and improved survival. Effective targeted delivery of chemotherapeutic drugs via VLP-based nano-vehicles is expected to address some of the primary challenges associated with cancer therapy. Our work in the growing field of virus nanoparticles opens avenues for engineering ligand-receptor-oriented targeted drug delivery vehicles for cancer therapy.

सार

कैंसर जैसी बीमारियों के इलाज के लिए टारगेटेड ड्रग डिलीवरी एक बड़ी चुनौती है, क्योंकि इलाज के मौजूदा तरीकों से जुड़े साइड इफ़ेक्ट होते हैं। एक अच्छे डिलीवरी सिस्टम की ज़रूरत है जो टारगेट साइट्स तक मॉलिक्यूल्स की डिलीवरी से जुड़ी चुनौतियों का सामना कर सके। कई वायरस के स्ट्रक्चरल प्रोटीन सही हालात में अपने आप वायरस जैसे पार्टिकल्स (VLPs) में बदल सकते हैं, जो उन्हें ड्रग एनकैप्सुलेशन और डिलीवरी के लिए असरदार नैनोकैरियर बनाता है।

हमने मेलेनोमा और कोलोरेक्टल कैंसर मॉडल्स में साइट-स्पेसिफिक पेप्टाइड फंक्शनलाइज़ेशन, हाइड्रोफोबिक ड्रग एनकैप्सुलेशन और टारगेटेड डिलीवरी के लिए एक टारगेटेड नैनो-व्हीकल बनाने के लिए एक इंसेक्ट नोडावायरस, फ्लॉक हाउस वायरस (FHV) के अंदरूनी फ़ायदों का इस्तेमाल किया है, जिससे रिसेप्टर-डायरेक्टेड नैनोथेरेप्यूटिक्स के लिए एक मॉड्यूलर फ्रेमवर्क बना है।

क्योंकि FHV में मैमेलियन सेल ट्रॉपिज़्म की कमी होती है, इसलिए थिओल-मैलेमाइड-बेस्ड कॉन्जुगेशन का इस्तेमाल करके, हमने पॉलीइथाइलीन ग्लाइकॉल बेस्ड क्रॉसलिंकर के ज़रिए FHV वायरस जैसे पार्टिकल्स (VLPs) से टारगेटिंग पेप्टाइड्स को जोड़ने का एक तरीका बनाया है। पेप्टाइड्स के जुड़ने से VLPs में ट्रॉपिज़्म की कमी दूर हो गई और वे कैंसर सेल्स पर ज़्यादा एक्सप्रेस होने वाले रिसेप्टर्स के साथ चुनिंदा रूप से जुड़ पाए। ऑप्टिमाइज़्ड कॉन्जुगेशन प्रोटोकॉल ने पार्टिकल्स की सिमिट्री और पूरी स्टेबिलिटी सुनिश्चित की। इसके अलावा, हाइड्रोफोबिक मॉलिक्यूल्स के लिए FHV की नेचुरल एफिनिटी का इस्तेमाल डॉक्सोरूबिसिन जैसी हाइड्रोफोबिक कीमोथेराप्यूटिक दवाओं को एनकैप्सुलेट करने के लिए किया गया है, Ca^{2+} कंसंट्रेशन के मॉड्यूलेशन के ज़रिए कैप्सिड को कुछ समय के लिए अस्थिर और फिर से स्थिर करके। इसके नतीजे में बदले हुए VLPs ने हाई ड्रग-लोडिंग कैपेसिटी और pH-रिस्पॉन्सिव

रिलीज़ बिहेवियर दिखाया, जिसमें फिजियोलॉजिकल pH पर कम से कम लीकेज और एसिडिक एंडोसोमल कंडीशन में तेज़ी से रिलीज़ हुआ।

इन मॉडिफाइड ड्रग एनकैप्सुलेटेड VLPs ने फ्री ड्रग से होने वाली तेज़ और बिना कंट्रोल वाली सेल डेथ की तुलना में, मुख्य रूप से एपोप्टोसिस के ज़रिए कंट्रोल्ड और लगातार साइटोटॉक्सिक असर दिखाया है। ड्रग-एनकैप्सुलेटेड VLPs म्यूरिन कोलोरेक्टल मॉडल में हाई एंटीकैंसर असर दिखाते हैं, जिससे ट्यूमर सप्रेशन होता है और सर्वाइवल बेहतर होता है। VLP-बेस्ड नैनो-व्हीकल्स के ज़रिए कीमोथेराप्यूटिक दवाओं की असरदार टारगेटेड डिलीवरी से कैंसर थेरेपी से जुड़ी कुछ मुख्य चुनौतियों का समाधान होने की उम्मीद है। वायरस नैनोपार्टिकल्स के बढ़ते क्षेत्र में हमारा काम कैंसर थेरेपी के लिए लिगैंड-रिसेप्टर-ओरिएंटेड टारगेटेड ड्रग डिलीवरी व्हीकल्स की इंजीनियरिंग के लिए रास्ते खोलता है।

TABLE OF CONTENT

S NO	TITLE	PAGE NO
1	CERTIFICATE	I
2	ACKNOWLEDGEMENT	II-III
3	ABSTRACT (ENGLISH/HINDI)	IV-VII
4	LIST OF FIGURES	IX-XI
5	LIST OF TABLES	XII
6	ABBREVIATIONS	XIII-XIV
7	**Chapter 1** INTRODUCTION	1-29
8	**Chapter 2** MATERIALS AND METHOD	30-38
9	**Chapter 3** ENGINEERING FLOCK-HOUSE VIRUS-LIKE PARTICLES FOR MELANOMA TARGETING AND QUANTIFICATION OF TARGETING MOIETIES	39-44
10	**Chapter 4** ENCAPSULATION OF HYDROPHOBIC DRUGS AND IN VITRO EVALUATION OF PEPTIDE-CONJUGATED DRUG-ENCAPSULATED VLPs	45-57
11	**Chapter 5** *IN VITRO* AND *IN VIVO* ASSESSMENT OF PEPTIDE-CONJUGATED VLPS FOR TARGETING COLORECTAL CANCER TUMORS	58-70
12	**Chapter 6** CONCLUSION AND FUTURE PERSPECTIVES	71-76
13	REFERENCES	77-101
14	ANNEXURES I (PUBLICATIONS)	102
15	ANNEXURES II (CURRICULUM VITAE)	103-106

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
1	Schematic representation of different modes of Viral entry into the host cells. A) Receptor mediated endocytosis. B) Fusion mediated entry. C) Pore mediated penetration	**3**
2	Illustration depicting different types of VLPs	**3**
3	A schematic showing the exposed Lysine residues for conjugation (red dots) on the FHV capsid. The inset shows the expected structural architecture of one icosahedral asymmetric unit of the capsid after chemical conjugation to the peptide via an SM(PEG)2 crosslinker. The FHV capsid structure is derived from the X-ray crystal structure of the iASU (PDB ID 4FTP)	**5**
4	Key Milestones in Cancer Therapeutics	**8**
5	Schematic overview of nanoparticle-based drug delivery systems	**9**
6	Some representative viruses being developed as nanoparticles for biomedical delivery	**15**
7	(A) Design of alpha MSH peptide (B) Schematic representation of peptide conjugation on the surface of S268K FHV VLP	**40**
8	Characterization of Conjugated and Unconjugated VLPs. A) Increment in molecular weight of FHV VLPs represents a shift of ~2kDa in SDS-PAGE. B) & C) Transmission Electron Microscopy images demonstrating the particle's uniform morphology and structural integrity post-conjugation	**42**
9	Characterization of Conjugated and Unconjugated VLPs. A) Nanoparticle tracking Analysis (NTA) displaying particle size and concentration of native and conjugated VLPs. B) NTA data showing a change in diffusion coefficient post-conjugation	**43**
10	Characterization of Conjugated and Unconjugated VLPs. Mass spectrometric analysis showing mass shift confirming peptide conjugation	**44**

11	An illustrative diagram representing the concept of active targeting of chemotherapeutic drugs by virus-like particles for cancer treatment. VLPs decorated with targeting ligands can specifically bind to receptors that are overexpressed on tumor cells, allowing localized drug delivery or internalization via receptor-mediated endocytosis	**46**
12	UV–Vis absorbance spectra of Doxorubicin, MSH_Dox VLP, and S268K	**47**
13	Schematic representing encapsulation strategy (A) Standard curve of doxorubicin generated from known concentrations of the drug for quantification (B) Transmission electron micrograph of Dox_α-MSH_S268K post encapsulation	**48**
14	Doxorubicin release from α-MSH_S268K–conjugated FHV VLPs. at pH 7.5 and pH 5.5. Data was collected at 2, 8, 24, 48, and 72 hrs., respectively, at 37oC	**49**
15	Confocal fluorescence microscopy images exhibiting cellular uptake and distribution in B16F10 control cells (top row), cells treated with free drug (middle row), and cells treated with Dox_ α-MSH_S268K VLPs.	**51**
16	(A): Dose-dependent cytotoxic evaluation of alphaMSH_S268K_DOX and free doxorubicin. The effective concentration of encapsulated and free doxorubicin was equal in each case.	**52**
	(B) Dose dependent cytotoxic evaluation of S268K VLPs at different concentrations	**53**
17	Flow cytometry-based apoptosis estimation in B16F10 cells post annexin- FITC staining - (i) Control cell population, (ii) S268K VLP, (iii) Cells incubated with free doxorubicin and (iv) tLyp-1_S268K_DOX, for 72h in each case	**55**
18	Stacked bar plots show distribution of viable, apoptotic, and necrotic cells in B16F10 cells after Annexin-FITC binding	**56**

19	Characterization of Conjugated and Unconjugated VLPs. A) Increment in molecular weight of FHV VLPs represents a shift of ~1.1 kDa in SDS-PAGE. B) & C) Transmission Electron Microscopy images demonstrating the particle's uniform morphology and structural integrity post-conjugation.	**59**
20	Chemical modification of VLPs for drug delivery. Nanoparticle tracking analysis of unconjugated and tLyP-1 conjugated S268K VLPs, (A) Size distribution of unconjugated and tLyp1 conjugated VLPs. (B) Change in diffusion coefficient upon conjugation of tLyp1 on VLP surface	**60**
21	Binding of tLyP-1 conjugated (lower panel) and unconjugated (upper panel) S268K VLPs to CT26 cells, visualized by confocal microscopy. The VLP capsid protein is labelled with a specific antibody and an Alexa-Fluor 488-conjugated secondary antibody	**61**
22	Internalization of free doxorubicin (upper panel) and VLP-encapsulated doxorubicin (lower panel) in CT26 cells	**62**
23	Dose-dependent cytotoxic evaluation of S268K VLPs at different concentrations	**63**
24	Dose dependent cytotoxic evaluation of tLyp-1_S268K_DOX and free doxorubicin. The effective concentration of encapsulated and free doxorubicin was equal in each case	**64**
25	Flow cytometry-based apoptosis estimation in CT26 cells post annexin-FITC staining - (i) Control cell population, (ii) Cells incubated with free doxorubicin, (iii) S268K VLP and (iv) tLyp-1_S268K_DOX, for 72h in each case	**65**
26	Stacked bar plots showing quantitative estimation of viable, apoptotic, and necrotic cells in CT26 cells after Annexin-FITC binding	**66**
27	Tumor-targeted drug delivery in colorectal cancer model (B) Change in tumor volume (C) Last day tumor volume	**68**
28	Tumor-targeted drug delivery in colorectal cancer model (A) % body weight change during the treatment course (B) median survival	**69**

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
1	Notable FDA-approved nanoparticle-based drug delivery systems	**10**
2	Comparison of limitations associated with conventional chemotherapy and advantages of virus-like particles over them.	**16**
3	Some VLP vaccines against different pathogens	**17**
4	List of different nanocarriers currently utilizing peptides for targeting and the corresponding targets	**20**
5	Genetically engineered VLPs and their applications	**23**
6	Some commonly used chemical conjugation strategies	**24**
7	List of equipment used during the course of research, along with the source company	**37**

LIST OF ABBREVIATIONS

Abbreviation	Full Form
α-MSH	Alpha-Melanocyte Stimulating Hormone
aa	Amino Acids
Ab	Antibody
Ag	Antigen
APC	Antigen-Presenting Cell
BSA	Bovine Serum Albumin
CMC	Critical Micelle Concentration
CT26	Murine Colorectal Carcinoma Cell Line 26
DC	Dendritic Cell
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl Sulfoxide
DOX	Doxorubicin
dsRNA	Double-Stranded Ribonucleic Acid
EPR	Enhanced Permeability and Retention Effect
ELISA	Enzyme-Linked Immunosorbent Assay
FHV	Flock House Virus
FHV VLPs	Flock House Virus-Like Particles
FITC	Fluorescein Isothiocyanate
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High-Performance Liquid Chromatography

HRP	Horseradish Peroxidase
IC50	Half Maximal Inhibitory Concentration
LC-MS	Liquid Chromatography–Mass Spectrometry
MC1R	Melanocortin-1 Receptor
MWCO	Molecular Weight Cut-Off
NHS	N-Hydroxysuccinimide
NPs	Nanoparticles
PAGE	Polyacrylamide Gel Electrophoresis
PBS	Phosphate Buffered Saline
PEG	Polyethylene Glycol
PEGylation	Surface Modification with Polyethylene Glycol
pH	Potential of Hydrogen
PLGA	Poly(Lactic-co-Glycolic Acid)
PVP	Polyvinylpyrrolidone
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
UV–Vis	Ultraviolet–Visible Spectroscopy
VLPs	Virus-Like Particles
WHO	World Health Organization