

**PROBING CONTRASTING FUNCTIONS OF VIRAL PROTEINS: AS
ASSEMBLIES FOR DRUG DELIVERY AND STIMULATORS FOR HOST
PROTEIN AGGREGATION**

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**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
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Probing contrasting functions of viral proteins: as
assemblies for drug delivery and stimulators for host
protein aggregation

by

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Dedicated to my family...

For their unconditional blessings, love and support

CERTIFICATE

This is to certify that the thesis titled “**Probing contrasting functions of viral proteins: as assemblies for drug delivery and stimulators for host protein aggregation**”, submitted by **Ms. Akanksha Saini** to the Indian Institute of Technology Delhi for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research she conducted, which has been prepared under my supervision and guidance following the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or whole to any other University for the award of any degree or diploma.

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Abstract

Historically, viruses have been significant contributors to morbidity and mortality in humans, which is recently exemplified by the global impact of the COVID-19 pandemic. Unraveling the virus lifecycle and developing effective therapeutics constitute critical research endeavors. Viruses hijack host cellular machinery for replication and assembly, with sustained viral infections posing a threat by compromising the host immune system, leading to potentially fatal outcomes. Recent studies indicate that viruses may facilitate the development of neurodegenerative disorders in humans. Examples include Herpes Simplex Virus-1 (HSV-1), which has been linked to Alzheimer's, Multiple Sclerosis (MS), and Parkinson's disease; Human T-cell Leukemia Virus type-1 (HTLV-1) which has been associated with tropical spastic paraparesis; and HIV, linked to the HIV-associated neurodegenerative disorder (HAND's). Thus, developing treatment strategies to prevent or slow down neurotropic virus-induced neurodegenerative disorders is a priority, which requires a deeper understanding of virus-host interaction pathways. Studying conserved sequences in viruses may be an essential tool for generating basic knowledge of such pathways. Such conserved sequences encode structural and non-structural viral proteins critical for various infection cycle stages. These sequences aid in identifying specific viruses and provide insights into conserved host-pathogen interaction techniques and can even serve as epitopes for vaccine development.

In the first part of the thesis work, we sought to elucidate the functional significance of the conserved pentapeptide sequence (VGGVV) identified in A β and analogous aggregation-prone viral proteins, such as the Virion Infectivity Factor (Vif-1) of Human Immunodeficiency Virus 1 (HIV-1). HIV plaques, detected in the cerebral tissue of the afflicted individuals, have been found to harbor beta-amyloid protein responsible for debilitating neurodegenerative Alzheimer's disease. A meticulous examination of Vif-1 was conducted to discern its propensity for inducing

aggregation in A β peptides. The study provided insights into the molecular mechanisms underpinning the correlation between viral infection and protein amyloid aggregation.

In the second part of the thesis, we explored the possibility of utilizing virus-like particles of Chikungunya Virus (CHIKV) as protein nanocontainers for delivery of foreign material to specific cell types. CHIKV VLPs were chemically modified to allow the conjugation of small, targeting peptides on the surface. The particles were subjected to a limited denaturation strategy to allow incorporation of fluorescent dye. The engineering strategies employed here resulted in the targeting of CHIKV VLPs to bone cells and breast carcinoma cells, as well as the deposition of the packaged cargo in target cells, indicating the potential of CHIKV nanocontainers for therapeutic delivery.

Thus, this study delves into both the disease-causing aspects of viruses and the therapeutic potential of virus nanocontainers. The findings of this work may contribute to advancing our understanding of virus-host interaction and pave the way for innovative therapeutic strategies involving virus-like particles.

ऐतिहासिक रूप से, वायरस मनुष्यों में रुग्णता और मृत्यु दर में अभिप्रायपूर्ण योगदानकर्ता रहे हैं, जिसका उदाहरण हाल ही में COVID-19 महामारी के वैश्विक प्रभाव से मिला है। वायरस के जीवनचक्र को उजागर करना और प्रभावी उपचार विज्ञान विकसित करना महत्वपूर्ण अनुसंधानिक प्रयास हैं। वायरस प्रतिकृति और संयोजन के लिए मेजबान सेलुलर मशीनरी का अपहरण कर लेते हैं, निरंतर वायरल संक्रमण मेजबान प्रतिरक्षा प्रणाली से समझौता करके खतरा पैदा करते हैं, जिससे संभावित घातक परिणाम होते हैं। हाल के अध्ययनों से संकेत मिलता है कि मनुष्यों को संक्रमित करने वाले वायरस न्यूरोडीजेनेरेटिव विकारों के विकास को बढ़ावा दे सकते हैं। उदाहरणों में अल्जाइमर, मल्टीपल स्केलेरोसिस (एमएस) और पार्किंसंस रोग से जुड़ा हर्पीस सिम्प्लेक्स वायरस-1 (एचएसवी-1); ट्रॉपिकल स्पास्टिक पैरापैरेसिस से जुड़ा ह्यूमन टी-सेल ल्यूकेमिया वायरस टाइप-1 (एचटीएलवी-1); और एचआईवी न्यूरोडीजेनेरेटिव डिसऑर्डर (एचएएनडी) से जुड़े वायरस एचआईवी शामिल हैं। इस प्रकार, न्यूरोट्रॉपिक वायरस-प्रेरित न्यूरोडीजेनेरेटिव विकारों को रोकने या धीमा करने के लिए उपचार रणनीतियों का विकास करना एक प्राथमिकता है, जिसके लिए वायरस-होस्ट इंटरैक्शन मार्गों की गहरी समझ की आवश्यकता होती है। ऐसे मार्गों के बारे में बुनियादी ज्ञान उत्पन्न करने के लिए वायरस में संरक्षित अनुक्रमों का अध्ययन एक आवश्यक साधन हो सकता है। ऐसे संरक्षित अनुक्रम विभिन्न संक्रमण चक्र चरणों के लिए महत्वपूर्ण संरचनात्मक और गैर-संरचनात्मक वायरल प्रोटीन को कूटबद्ध करते हैं। ये अनुक्रम विशिष्ट वायरस की पहचान करने में सहायता करते हैं और संरक्षित मेजबान-रोगजनक इंटरैक्शन तकनीकों में अंतर्दृष्टि प्रदान करते हैं, और यहां तक कि टीका विकसित करने के लिए एपिटोप के रूप में भी काम कर सकते हैं।

थीसिस कार्य के पहले भाग में, हमने A β में पहचाने गए संरक्षित पेंटापेप्टाइड अनुक्रम (VGGVV) और अनुरूप एकत्रीकरण-प्रवण वायरल प्रोटीन जैसे मानव इम्युनोडेफिशिएंसी वायरस 1 (एचआईवी-1) के विरियन संक्रामक कारक (वीआईएफ-1) के कार्यात्मक महत्व को स्पष्ट करने की कोशिश की है। पीड़ित व्यक्तियों के मस्तिष्क के ऊतकों में पाए जाने वाले एचआईवी प्लाक में न्यूरोडीजेनेरेटिव अल्जाइमर रोग को कमजोर करने के लिए जिम्मेदार बीटा-एमिलॉइड प्रोटीन पाया गया है। A β पेप्टाइड्स में एकत्रीकरण को प्रेरित करने की इसकी प्रवृत्ति को समझने के लिए वीआईएफ-1 की सावधानीपूर्वक जांच की गई। अध्ययन ने वायरल संक्रमण और प्रोटीन अमाइलॉइड एकत्रीकरण के बीच संबंध को रेखांकित करने वाले आणविक तंत्र में अंतर्दृष्टि प्रदान की।

थीसिस के दूसरे भाग में, हमने विशिष्ट प्रकार की कोशिकाओं तक बाहरी पदार्थ पहुंचाने के लिए चिकनगुनिया वायरस (CHIKV) के वायरस जैसे कणों को प्रोटीन नैनोकंटेनर के रूप में उपयोग करने की

संभावना का पता लगाया। सतह पर छोटे, लक्षित पेप्टाइड्स के संयुग्मन की अनुमति देने के लिए CHIKV वीएलपी को रासायनिक रूप से संशोधित किया गया था। फ्लोरोसेंट डाई को शामिल करने की अनुमति देने के लिए कणों को एक सीमित विकृतीकरण कार्यनीति के अधीन किया गया था। यहां नियोजित इंजीनियरिंग कार्यनीतियों के परिणामस्वरूप CHIKV वीएलपी को हड्डी कोशिकाओं और स्तन कार्सिनोमा कोशिकाओं को लक्षित किया गया, साथ ही लक्षित कोशिकाओं में पैक किए गए कार्गो का जमाव हुआ, जो चिकित्सीय वितरण के लिए CHIKV नैनोकंटेनर्स की क्षमता का संकेत देता है।

इस प्रकार, यह अध्ययन वायरस के रोग पैदा करने वाले पहलुओं और वायरस नैनोकंटेनरों की चिकित्सीय क्षमता दोनों पर प्रकाश डालता है। इस कार्य के निष्कर्ष वायरस-मेजबान संपर्क की हमारी समझ को आगे बढ़ाने में योगदान दे सकते हैं और वायरस जैसे कणों को शामिल करने वाली नवीन चिकित्सीय कार्यनीतियों का मार्ग प्रशस्त कर सकते हैं।

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LIST OF ABBREVIATIONS

A280	Absorbance measured at 280 nm
APS	Ammonium per sulfate
Å	Angstrom
~	Approximately
A β	Beta Amyloid
AIDS	Acquired Immunodeficiency Syndrome
APP	Amyloid Precursor Protein
a.u.	Arbitrary Units
CD	Circular dichroism
°C	Degree Celsius
CNS	Central Nervous System
C _p	Heat Capacity at constant pressure
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
DSC	Differential Scanning Calorimetry
e.g.	Exempli gratia, or “for example”
λ_{max}	Fluorescence emission maxima
g	Gram
HIV-1	Human Immunodeficiency Virus type 1
HSV-1	Herpes Simplex Virus type 1
hrs	Hour/hours
i.e.	I d est, or “that is”
kb	Kilobase pair
kDa	K ilodalton
μg	Microgram
μl	Microliter
mdeg	Milli degrees
mg	Milligram
ml	Milliliter
Mm	Millimolar
min	Minute
M	Molar
MW	Molecular weight
DAPI	4',6-diamidino-2-phenylindole
ng	Nanogram
nm	Nanometer
NCBI	National Center for Biotechnology Information
DMEM	Dulbecco's Modified Eagle Medium
%	Percent
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
RFU	Relative Fluorescence Units
rpm	Revolutions per minute
RT	Room Temperature
s	Second
SDS	Sodium dodecyl sulphate
SP	Slow Progressors of HIV-1
h	hours
T _m	Melting Temperature
EGFP	Enhanced green fluorescent protein
Trp	Tryptophan

vs.	Versus
VGGVV	N'-Val-Gly-Gly-Val-Val-C' peptide stretch
Vif	Virion Infectivity Factor
HRP	Horseradish peroxidase
CHIKV	Chikungunya virus
RNA	Ribonucleic acid
T	Triangulation number
RRV	Ross River Virus
VEEV	Venezuelan Eastern Equine Encephalitis Virus
SFV	Semliki Forest Virus
SINV	Sindbis Virus
NSP	Non-Structural Protein
Tat	Trans-Activator of Transcription
VLP	Virus Like Particle
TBST	Tris Buffered Saline with Tween
NaCl	Sodium Chloride
Tris	Trisaminomethane
HEK	Human Embryonic Kidney
VGGVV	N'-Val-Gly-Gly-Val-Val-C' peptide stretch
NaAcetate	Sodium Acetate
PBS	Phosphate Buffered Saline
SEC	Size Exclusion Chromatography
CDNA	complementary DNA
PVDF	Polyvinylidene difluoride
VMD	Visual Molecular Dynamics
SD	Standard Deviation
WGA	Wheat Germ Agglutinin
MD	Molecular Dynamics
DMEM	Dulbecco's Modified Eagle's Medium
FBS	Fetal Bovine Serum
SulfoB	Sulforhodamine B
KCl	Potassium Chloride
MgCl	Magnesium Chloride
EGFP	Enhanced Green Fluorescent Protein
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
Ct	Cyclic Threshold
HRP	Horse Radish Peroxidase
rpm	Revolutions per minute
TEM	Transmission Electron Microscopy
SW982	Synoviocytes
MDAMB231	M.D. Anderson and MB stands for Metastasis Breast cancer
SHSY5Y	SK-N-SH neuroblastoma cell line
HCE	Human corneal epithelial cell
ND	Neurodegenerative Disorder
AD	Alzheimer's Disease
PD	Parkinson's Disease