Unveiling the Structures of N0-P Complex in High-Risk Paramyxoviruses: Development of Small Inhibitors against Paramyxoviral Infections

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Background

- Paramyxoviral (PMV) infections, including those caused by Nipah Virus (NiV), Hendra Virus (HeV), Langya Virus (LayV), and Newcastle Disease Virus (NDV), pose significant threats to human and animal health due to high mortality rates and the lack of approved therapeutics.
- One of the major concerns is these viruses are found in globally and right now Paramyxoviridae family is divided into 9 Subfamilies, 23 Genera, 153 Species according to ICTV.

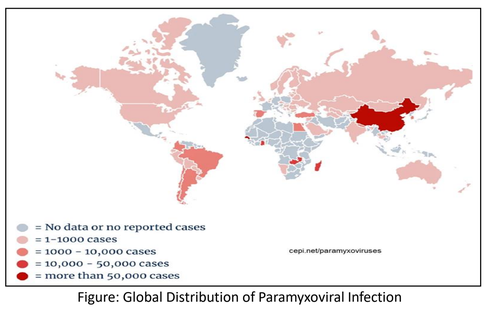

Figure: Global Distribution of Paramyxoviral Infection

- The PMV RNA genome is encapsidated by the viral nucleoprotein. Preceding encapsidation, PMVs require a constant supply of unassembled N (N0) units facilitated by complex formation with its viral chaperone, P.
- The encapsidation of the RNA in this ribonucleoprotein structure, known as the nucleocapsid, prevents the annealing of positive and negative RNA strands into double-stranded RNA structures, as well as the folding of genomic RNA into secondary RNA structures, which helps to prevent recognition by innate immune receptors. It also protects the genome from degradation by nucleases.
- **Inhibiting the formation of the N0-P complex is a promising therapeutic strategy**

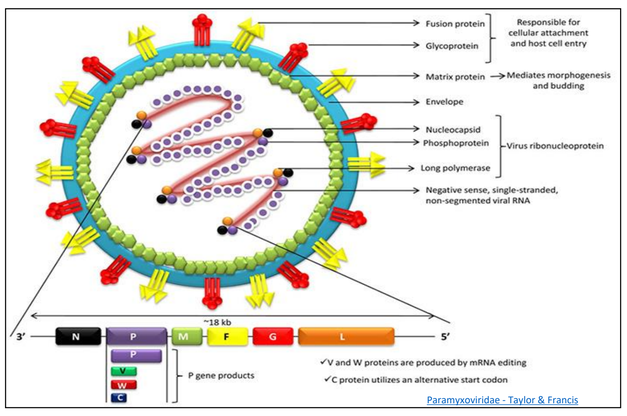

Figure: Genomic and Proteomic Organization of Paramyxoviral Infection

Objectives

To use protein crystallography to resolve the interface of N:P complex of some high-risk paramyxoviruses

To Investigate the Conserved and diverged region among the unveiled N0-Ps and comparison of Nipah N0-P

To design and screen small inhibitors against N0-P complexes

To assess the efficacy and cytotoxicity of the screened compounds by cell-based assay

Methodology

Section 1: Structural Determination

1. Plasmids will be transformed in E. coli for transformation
2. Protein Expression will be induced in E. coli
3. Desired protein will be purified by His-tag Affinity Chromatography and size-exclusion chromatography
4. Protein Crystallization will be performed by crystal screening plate
5. Crystal will be sent to Australian Synchrotron
6. Received Data will be processed for structural analysis

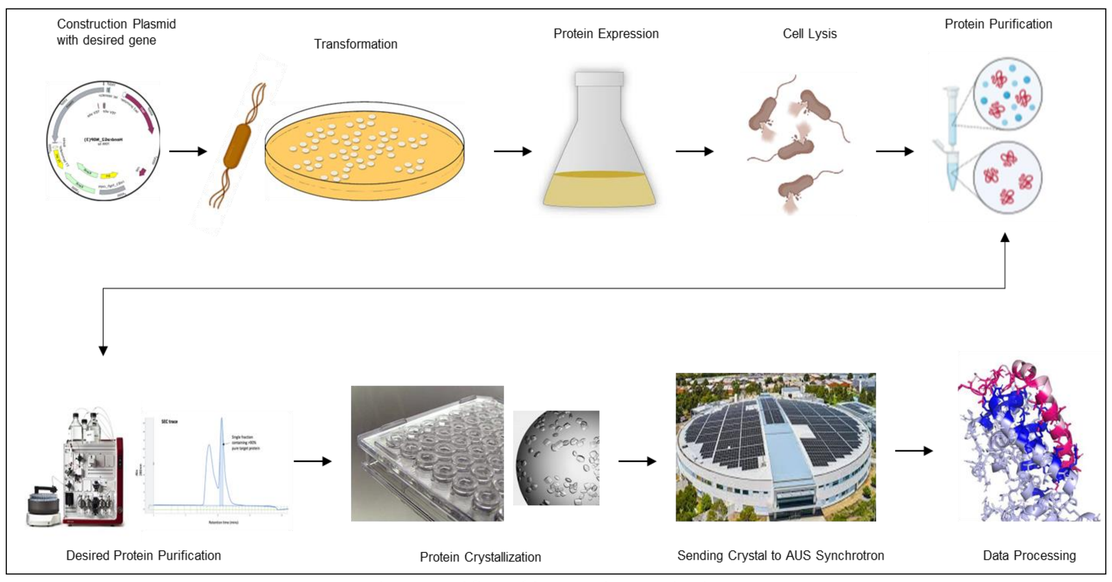

Figure: Workflow of Structural Determination

Section 2: Designing and Screening Inhibitors

1. Ligand library will be collected from different drug bank database and designed if necessary
2. All compounds will be screened by molecular docking and dynamic simulation
3. Best binding compounds will be synthesised for in vitro screening
4. Different binding assay like EMSA, FP will be performed
5. Cell based cytotoxicity assay and FRNT for efficacy test will be performed
6. Successful inhibitors will be taken for complexing studies with protein

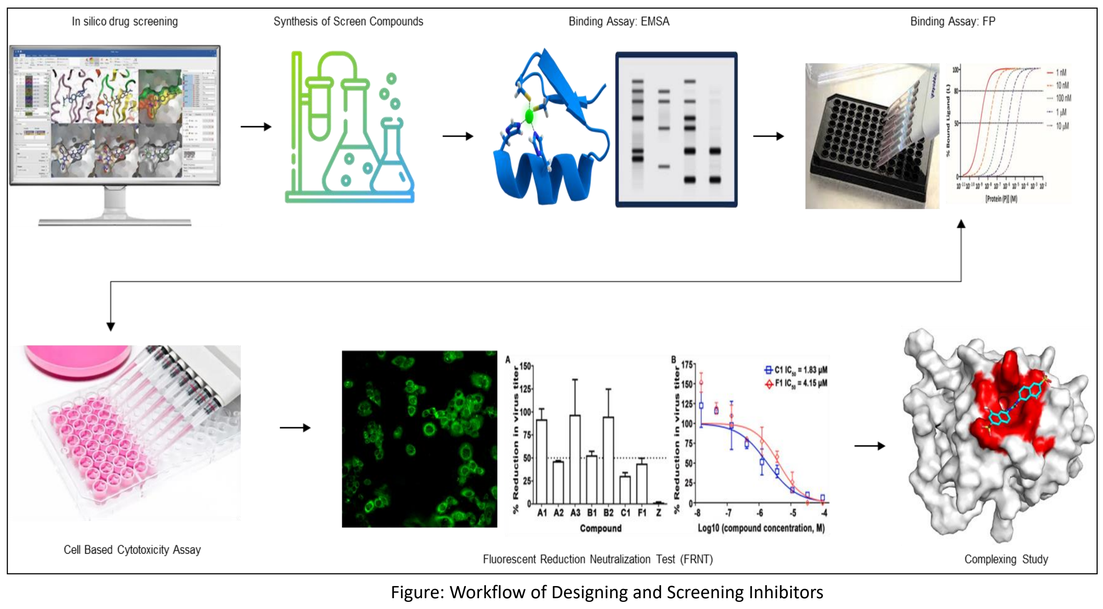

Figure: Workflow of Designing and Screening Inhibitors

Results

Ten Sequences have been selected for resolving N0-P structures from high-risk paramyxoviruses and 1 sequence from Bornavirus

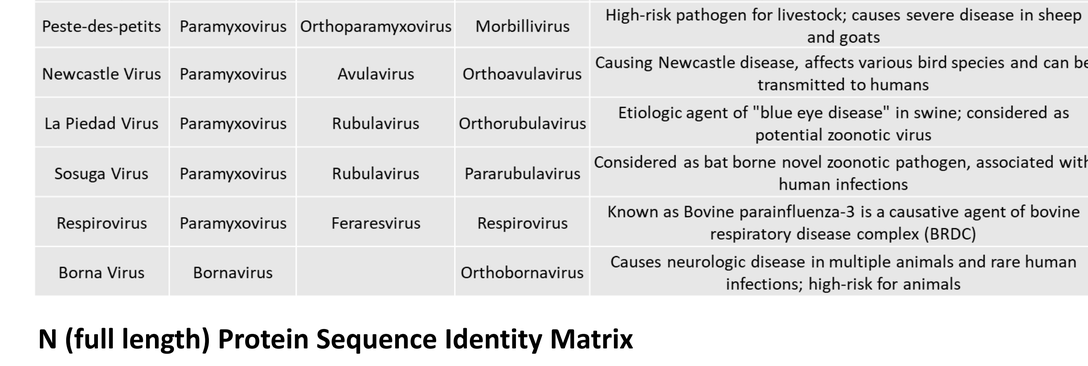

Figure: Phylogenetic Determination of Selected Paramyxoviruses

Evaluating Potential Risks of 10 selected Paramyxoviruses: Aiming to Design broad spectrum Inhibitors

Name of Virus	Family	Subfamily	Genus	Potential Risk
Hendra G2	Paramyxovirus	Orthoparamyxovirus	Henipavirus	High-risk pathogen (Risk Group 4); very close to Nipah virus
Cedar	Paramyxovirus	Orthoparamyxovirus	Henipavirus	Non-pathogenic; used as a model virus for henipavirus research in BSL-2 facilities
Angavokely	Paramyxovirus	Orthoparamyxovirus	Henipavirus	Potential zoonotic pathogen; newly emerged bat-borne virus
Langya	Paramyxovirus	Orthoparamyxovirus	Parahenipavirus	Potential zoonotic pathogen; has caused clinical cases in humans in China
Jeilong mastomysis	Paramyxovirus	Orthoparamyxovirus	Jeilongvirus	Emerging bat-borne paramyxovirus with unique N0-P sequence; potential zoonotic risk
Peste-des-petits	Paramyxovirus	Orthoparamyxovirus	Morbillivirus	High-risk pathogen for livestock; causes severe disease in sheep and goats
Newcastle Virus	Paramyxovirus	Avulavirus	Orthoavulavirus	Causing Newcastle disease, affects various bird species and can be transmitted to humans
La Piedad Virus	Paramyxovirus	Rubulavirus	Orthorubulavirus	Etiologic agent of "blue eye disease" in swine; considered as potential zoonotic virus
Sosuga Virus	Paramyxovirus	Rubulavirus	Pararubulavirus	Considered as bat borne novel zoonotic pathogen, associated with human infections
Respirovirus	Paramyxovirus	Feraresvirus	Respirovirus	Known as Bovine parainfluenza-3 is a causative agent of bovine respiratory disease complex (BRDC)
Borna Virus	Bornavirus		Orthobornavirus	Causes neurologic disease in multiple animals and rare human infections; high-risk for animals

N (full length) Protein Sequence Identity Matrix

Seq	Nipah	Hendra	Cedar	Angavokely	Langya	Mastomys	Peste-des-petits	Newcastle Virus	La piedad	Sosuga	Respirovirus	Borna
Nipah	100.00%	90.40%	59.20%	46.00%	48.20%	34.50%	30.10%	23.70%	24.60%	26.30%	20.90%	7.30%
Hendra	90.40%	100.00%	59.00%	45.80%	46.40%	34.60%	30.40%	23.70%	23.90%	26.10%	20.50%	7.50%
Cedar	59.20%	59.00%	100.00%	48.10%	45.10%	33.90%	30.00%	24.40%	25.20%	27.20%	22.90%	9.70%
Angavokely	46.00%	45.80%	48.10%	100.00%	44.70%	33.00%	31.70%	22.90%	26.10%	26.00%	20.90%	8.50%
Langya	48.20%	46.40%	45.10%	44.70%	100.00%	34.70%	30.60%	22.30%	24.90%	24.90%	21.10%	7.70%
Mastomys	34.50%	34.60%	33.90%	33.00%	34.70%	100.00%	32.70%	22.60%	22.90%	22.10%	22.00%	8.10%
peste-des-petits	30.10%	30.40%	30.00%	31.70%	30.60%	32.70%	100.00%	22.20%	23.10%	22.60%	19.70%	8.70%
Newcastle Virus	23.70%	23.70%	24.40%	22.90%	22.30%	22.60%	22.20%	100.00%	30.40%	31.90%	20.00%	7.70%
La Piedad	24.60%	23.90%	25.20%	26.10%	24.90%	22.90%	23.10%	30.40%	100.00%	45.80%	18.20%	8.20%
Sosuga virus	26.30%	26.10%	27.20%	26.00%	24.90%	22.10%	22.60%	31.90%	45.80%	100.00%	19.50%	8.90%
Respirovirus	20.90%	20.50%	22.90%	20.90%	21.10%	22.00%	19.70%	20.00%	18.20%	19.50%	100.00%	10.40%
Borna	7.30%	7.50%	9.70%	8.50%	7.70%	8.10%	8.70%	7.70%	8.20%	8.90%	10.40%	100.00%

P(1-50) Protein Sequence Identity Matrix

Seq	Nipah	Hendra	Cedar	Angavokely	Langya	Mastomys	Peste-des-petits	Newcastle Virus	La piedad	Sosuga	Respirovirus	Borna
Nipah	100.00%	94.00%	50.00%	58.00%	41.80%	42.80%	11.30%	22.80%	13.30%	12.20%	1.70%	7.00%
Hendra	94.00%	100.00%	52.00%	62.00%	43.60%	44.60%	11.30%	22.80%	13.30%	14.00%	3.40%	8.70%
Cedar	50.00%	52.00%	100.00%	46.00%	23.60%	35.70%	13.20%	17.50%	15.00%	21.00%	1.70%	7.00%
Angavokely	58.00%	62.00%	46.00%	100.00%	43.60%	44.60%	15.00%	14.00%	13.30%	17.50%	6.80%	7.00%
Langya	41.80%	43.60%	23.60%	43.60%	100.00%	37.70%	11.10%	20.70%	8.70%	14.80%	1.80%	11.50%
Mastomys	42.80%	44.60%	35.70%	44.60%	37.70%	100.00%	11.10%	27.40%	18.50%	19.60%	5.70%	11.50%
peste-des-petits	11.30%	11.30%	13.20%	15.00%	11.10%	11.10%	100.00%	10.90%	12.00%	9.00%	7.20%	1.80%
Newcastle Virus	22.80%	22.80%	17.50%	14.00%	20.70%	27.40%	10.90%	100.00%	22.20%	23.00%	7.50%	9.60%
La Piedad	13.30%	13.30%	15.00%	13.30%	8.70%	18.50%	12.00%	22.20%	100.00%	16.90%	11.10%	8.90%
Sosuga virus	12.20%	14.00%	21.00%	17.50%	14.80%	19.60%	9.00%	23.00%	16.90%	100.00%	8.00%	3.70%
Respirovirus	1.70%	3.40%	1.70%	6.80%	1.80%	5.70%	7.20%	7.50%	11.10%	8.00%	100.00%	7.50%
Borna	7.00%	8.70%	7.00%	7.00%	11.50%	11.50%	1.80%	9.60%	8.90%	3.70%	7.50%	100.00%

This study is now in the initial stages. Although, The Plasmid constructs for N0-P structures has been received and laboratory experiments have been started already.

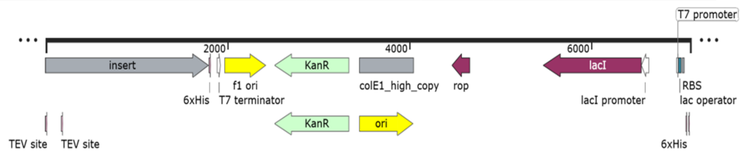

Figure: Hendra G2 N0-P Construct

Conclusion

This research aims to unveil the N0-P structures of some high-risk PMVs using recombinant protein expression and X-ray crystallography methods. Structural analysis will identify conserved regions suitable for therapeutic drug design and offer viable solutions for improving the response to emerging infectious diseases.

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