UNIVERSITI PUTRA MALAYSIA

NUCLEOCAPSID AND MATRIX PROTEINS OF NIPAH VIRUS PRODUCED IN Pichia pastoris AND THEIR INTERACTION

NARCISSE MARY A/P SITHER JOSEPH VESUDIAN

FBSB 2016 12

NUCLEOCAPSID AND MATRIX PROTEINS OF NIPAH VIRUS PRODUCED IN *Pichia pastoris* AND THEIR INTERACTION

By

NARCISSE MARY A/P SITHER JOSEPH VESUDIAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

February 2016

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

NUCLEOCAPSID AND MATRIX PROTEINS OF NIPAH VIRUS PRODUCED IN *Pichia pastoris* AND THEIR INTERACTION

By

NARCISSE MARY A/P SITHER JOSEPH VESUDIAN

February 2016

Chairman : Professor Tan Wen Siang, PhD
Faculty : Biotechnology and Biomolecular Sciences

Nipah Virus (NiV) is a zoonotical pathogen belonging to the family of *Paramyxoviridae* and genus *Henipavirus.* Its natural reservoir is believed to be fruit bats, *Pteropus sp*. The outbreaks of NiV in Malaysia, India and Bangladesh recorded high fatality rate imposing a major health risk worldwide. It is classified under Biosafety level 4 pathogen and the production of immunological reagents for assays using the inactivated virus requires BSL 4 facilities which are costly and limited. Recombinant proteins produced in *Escherichia coli* served as an alternative, however being a prokaryotic expression system, disadvantages such as lack of post-translational modifications are unavoidable. The nucleocapsid (N) and matrix (M) proteins produced in *E. coli* expression system were different from the native viral proteins. Thus, the aim of this study was to produce the N and M proteins of NiV in a yeast system, *Pichia pastoris,* and to study their interaction. The interaction of N and M proteins will help to reveal the role of these proteins in the production of VLPs. The N protein was successfully expressed in *P. pastoris* and purified using sucrose density ultracentrifugation but was found to be truncated at the C-terminal end. However the recombinant nucleocapsid protein assembled into tightly packed long helical structures as long as 1.5 µm. The M protein of NiV was expressed in *P. pastoris* and purified using an Immobilized Metal Affinity Chromatography (IMAC) column. The M protein also assembled into large virus-like particles resembling the native virus. However, due to the poor yield and purity of the M protein produced in *P. pastoris*, an *in-silico* method called docking was chosen to study the interaction of M and N proteins. The 3-dimensional structures of the N and M proteins of NiV are not available in the Protein Data Bank (PDB) for docking analysis and there are no similar structures available. Therefore, the structures of the two proteins were predicted based on an *ab initio* method using the I-TASSER web server. The predicted 3D structures generated using ITASSER had a confidence score (C-score) of -0.82 and 0.56 for N and M proteins, respectively. The final models also had a low root-mean-square deviation (RMSD) (9 Å for N and 5.3 Å for M protein) which shows the reliability of the predicted structure. The interaction of N and M proteins was analysed using a molecular docking employing the PatchDock method and refined with the FireDock algorithm. The two proteins were found to interact well

with a binding energy of −13.09 kcal/mol. However to validate the docking results, five peptides corresponding to the interacting residues in the M protein were synthesized to study the interaction using an *in-vitro* assay. The instability of the N protein lead to a simple and less complicated method of interaction study, using fluorescence spectroscopy analysis.. The fluorescence quenching assay demonstrated that peptides M2 and M4 show significant interactions with the NiV N protein with dissociation constant (K_d) values of 12 μM and 17 μM, respectively. This indicates that the M protein interacts with the N protein and proves the M and N proteins interact during the VLP formation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGEKSPRESAN PROTEIN NUKLEOKAPSID DAN MATRIKS VIRUS NIPAH DALAM *Pichia pastoris* DAN KAJIAN INTERAKSI

Oleh

NARCISSE MARY A/P SITHER JOSEPH VESUDIAN

Februari 2016

Pengerusi : Profesor Tan Wen Siang, PhD
Fakulti : Bioteknologi dan Sains Biomolekul

Virus Nipah (NiV) adalah patogen zoonotikal dalam family *Paramyxoviridae* dan genus Henipavirus. NiV dipercayai berasal dari kelawar dari genus *Pteropus sp*. NiV telah menyebabkan wabak di Malaysia, India dan Bangladesh dengan mencatatkan kadar kematian yang tinggi. Ia dikelaskan sebagai patogen 'Biosafety (BSL)-4' dan penyelidikan virus tersebut memerlukan makmal dengan kemudahan BSL-4 yang mahal dan terhad. Protein rekombinan yang telah dihasilkan dalam *Escherichia coli* telah menjadi alternatif untuk kajian NiV, bagaimanapun sebagai satu sistem prokariot, kekurangan seperti modifikasi pasca translasi tidak dapat dielakkan. Protein nukleokapsid (N) dan matriks (M) yang dihasilkan dalam sistem *E. coli* adalah sangat berbeza dari protein virus sebenar. Oleh itu, tujuan kajian ini adalah untuk menghasilkan protein N dan M dalam sistem yis, *Pichia pastoris*, dan mengkaji interaksi protein-protein tersebut. Protein N telah berjaya diekspres dalam *P. pastoris* dan dipulihkan melalui proses ultra-pengemparan menggunakan sukrosa tetapi protein tersebut telah didapati dihadam pada hujung karboksi-C. Namun, protein rekombinan tersebut telah mebentuk struktur heliks padat yang sangat panjang (1.5 μm). Protein M pula telah diekspres dalam *P. pastoris* dan ditulenkan menggunakan sistem afiniti 'Metal Affinity Chromatography (IMAC)'. M protein juga telah bergabung sendiri membentuk partikel virus besar menyerupai virus asli. Walau bagaimanapun disebabkan oleh hasil yang tidak mencukupi dan ketulenan protein M yang dihasilkan menggunakan *P. pastoris* tidak memuaskan, kaedah *in-silico* telah dipilih untuk mengkaji interaksi protein M dan N. Struktur 3 dimensi (3D) protein N dan M telah dihasilkan menggunakan program ITASSER yang meramalkan struktur 3D protein berdasarkan jujukan asid amino. Oleh kerana tidak ada struktur yang menyerupai protein M dan N di Protein Data Bank (PDB) untuk dirujuk, struktur kedua-dua protein tersebut telah diramal dengan menggunakan kaedah *ab-initio* menggunakan pengkalan pesawang I-TASSER. Struktur 3D yang telah dijana menggunakan ITASSER mempunyai nilai keyakinan (C-skor) yang tinggi iaitu sebanyak -0,82 untuk protein N dan 0.56 untuk protein M. Model-model tersebut mempunyai 'root-mean-square deviation (RMSD)' yang memuaskan iaitu 9 Å untuk protein N dan 5.3 Å untuk protein M. Ini menunjukkan kebolehpercayaan struktur yang telah diramalkan. Interaksi protein N dan M dianalisis menggunakan

‘docking’ molekul melalui program PatchDock dan diperbetulkan dengan program FireDock. Kedua-dua protein didapati berinteraksi dengan baik dengan tenaga pengikatan sebanyak -13,09 kcal/mol. Walau bagaimanapun, lima peptida yang sepadan dari protein M yang berinteraksi dengan protein N telah disintesis untuk mengesahkan keputusan ‘docking’ secara *in-vitro*. Ketidakstabilan protein N telah membawa kepada penggunaan satu kaedah yang mudah dan kurang rumit untuk kajian interaksi iaitu spektroskopi ‘flourescence’. Analisa tersebut menunjukkan bahawa peptida M2 dan M4 menunjukkan interaksi yang memuaskan dengan protein N dengan nilai pemalar pengasingan (K_d) 12 μM dan 17 μM, masing-masing.

ACKNOWLEDGEMENTS

I first and foremost would like to thank God for granting me the courage that was necessary to complete this work after many years of struggle. Only because of his mercy and showers of blessings throughout my research, I was able to complete the research successfully.

I would like to extend my appreciation to my supervisor, Prof Dr. Tan Wen Siang for his guidance and kind patience throughout my study. With his psychological motivations and invaluable advices, he helped me overcome many obstacles I faced during my study.

I would also like to convey my gratitude to all the wonderful members of my Supervisory Committee, Assoc. Prof. Dr. Norazizah Shafee, Dr. Ho Kok Lian, Dr. Tan Chon Seng and Prof. Dr. Tey Beng Ti for their help and support in completing my work. Their contributions are very much appreciated. My warmest thanks to Dr. Bimo Tejo, without his precious support it would not be possible to complete this thesis.

Special mention is reserved for all my lab mates and friends, Yap Wei Boon, Jason Lee Khai Wooi, Azira, Yong Chean Yeah, Goh Zee Hong and all the members of Lab 134, for their endless support and great friendship provided from the beginning until the very end.

I am infinitely grateful to the treasure of my life, my dear husband for all the patience and love he has for me. His continuous encouragement, advice and support helped me to complete this thesis.

I certify that a Thesis Examination Committee has met on 16 February 2016 to conduct the final examination of Narcisse Mary a/p Sither Joseph on her thesis entitled "Nucleocapsid and Matrix Proteins of Nipah Virus Produced in *Pichia pastoris* and Their Interaction" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Muhajir bin Hamid, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Noorjahan Banu binti Mohammed, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Parameswari a/p Namasivayam, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Shin-Ichiro Suye, PhD
Professor
University of Fukui
Japan
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 June 2016

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Tan Wen Siang, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Norazizah Shafee, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Ho Kok Lian, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Tan Chon Seng, PhD
Research Officer
Malaysian Agricultural Research and Development Institute
Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been duly referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- Intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- Written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before the thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/ fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism software.

Signature: _____ Date: _____

Name and Matric No: Narcisse Mary A/P Sither Joseph Vesudian, GS 20846

Declaration by Members of Supervisory Committee

This is to confirm that:

- The research and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Professor Dr. Tan Wen Siang

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Norazizah Shafee

Signature: _____
Name of Member of Supervisory Committee: Dr. Ho Kok Lian

Signature: _____
Name of Member of Supervisory Committee: Dr. Tan Chon Seng

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

aa	amino acids
AAHL	CSIRO Australian Animal Health Laboratory
Abs	absorbance
APS	ammonium persulfate
Bp	base pair
BSA	bovine serum albumin
BCIP	5-bromo-4-chloro-3'-indolyl phosphate
°C	degree Celsius
CBB	coomasie brilliant blue
C-terminal	carboxyl-terminal
dH_2O	distilled water
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dNTPs	deoxynucleotide triphosphates
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
h	hour
HeV	Hendra virus
ICTV	International Committee on Taxonomy of Viruses
IMAC	immobilized metal affinity chromatography
IgG	immunoglobulin G
IgM	immunoglobulin M
IPTG	isppropylthio-β-d-galactoside
kDa	kilodalton
L	liter
LB	luria bertani
M	molar

mA	miliampere
mg	milligram
mg/mL	milligram per milliliter
mg/L	milligram per liter
min	minute
mL	milliliter
mM	milimolar
mRNA	messenger RNA
NaCl	sodium chloride
NaoH	sodium hydroxide
$NaPO_4$	sodium phosphate
NBT	nitro-blue tetrazolium chloride
NC	nitrocellulose
NiV	Nipah virus
N-termial	amino-terminal
NMR	nuclear magnetic resonance
OD	optical density
PCR	polymerase chain reaction
PAGE	polyacrylamide gel
pH	*puissance hydrogen*
PMSF	phenylmethylsulphonyl fluoride
p-npp	p-nitrophenylphosphate
RNA	ribonucleic acid
RT	room temperature
RT-PCR	reverse transcriptase-polymerase chain reaction
rpm	revolution per minute
S	second
SDS	sodium dodecyl sulfate
TBS	tris buffer saline

TAE	Tris-acetate-EDTA buffer
TEM	transmission electron microscope
TEMED	tetramethyenediamine
µg	microgram
µM	micromolar
UA	uranyl acetate
V	volt
v/v	volume per volume
w/v	weight per volume
W	watt
x g	centrifugal force (multiply gravity)

Amino acids abbreviations

Amino acid	Three letter abbreviation	One letter abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	T
Valine	Val	V

CHAPTER 1

INTRODUCTION

Nipah virus (NiV) is a zoonotic pathogen that causes febrile encephalitis and respiratory disease in humans, pigs and many domestic animals such as cats and dogs (Field *et al*., 2001; Chua *et al.*, 2000). It belongs to the family *Paramyxoviridae* and is grouped into the newly emerged genus *Henipavirus*. The virus was initially isolated and identified during an outbreak of febrile encephalitis in Malaysia in 1998 and 1999. Eventually, NiV reoccurred in many sporadic outbreaks in India and Bangladesh, and it exhibited an extremely high mortality rate (Luby *et al*., 2009). A fruit bat from the genus *Pteropus* was identified as the reservoir host of the virus. High fatality rate and broad species tropism make NiV a biological threat that constitutes a major health risk worldwide.

NiV particles are pleomorphic in shape and contain a single-stranded, negative-sense RNA genome encoding six major structural proteins that are essential for particle assembly and budding, namely nucleocapsid (N), phospho-(P), matrix (M), fusion (F), attachment (G) and large (L) proteins. The structural proteins of paramyxoviruses assemble in a coordinated way via specific interactions during viral assembly and budding, which produces infectious viral particles. During viral assembly, the N protein subunits encapsidate the viral genome RNA and form a helical structure called the core protein. The N potein, not only protects the viral genome but also plays a key role during viral transcription and replication (Moeller *et al*., 2012). The core protein binds with the RNA-dependent RNA polymerase complex that is composed of the P and L protein subunits via specific N-P interactions to form a complex called the ribonucleoprotein complex (RNP). The N–P interactions for the members of *Paramyxoviridae* including NiV have been investigated. The interaction study of P protein of NiV with its N protein revealed the presence of more than one N binding sites on the P protein; the N–terminal (amino acids 3–220) and the C-terminal (amino acids 636–709) ends (Chan *et al*. 2004).

The RNP complex which is vital for viral transcription and genome replication is then packaged within a lipid envelope derived from the host plasma membrane containing the surface proteins; F and G, which are present on the outer surface of the viral envelope (Eaton *et al*., 2006). As is the case for most negative-strand RNA viruses, the association of the NiV RNP with the viral membrane is mediated by the M protein. The M protein is a major structural protein that plays a multi-functional role during the viral assembly and budding. M proteins coordinate the viral particle formation by assembling different viral components and organizing viral budding.

The structure and function of the structural proteins of paramyxoviruses, including the M protein, have been studied extensively in recent years. However, research on NiV M protein has progressed far less compared to research of other members in the family, such as Sendai, measles and respiratory syncytial virus (RSV) viruses. This is

due to the classification of NiV as a Biosafety level (BSL)-4 pathogen due to its high fatality rate and the lack of vaccines or post-exposure therapeutics. The need for high-containment lab facilities limits the future research and makes the ability to work on this virus a challenge. Therefore, the viral assembly and budding pathways of NiV are still not fully understood.

Recent advancement of molecular techniques and recombinant protein technology enables researchers to produce non-infectious viral particles that are physically and morphologically similar to their native virus, termed virus-like particles (VLPs). The production of VLPs is an opportunity to explore the structure and function of the structural protein individually. VLP formation of an enveloped virus is highly dependent on the M protein because it acts as bridges between the envelope glycoproteins and the ribonucleoprotein complexes to self-assemble into infectious viral particles as proven in Newcastle disease virus (NDV) and also NiV (Lamb and Parks, 2007). However, the precise mechanism or the interacting domain of the N and M proteins of paramyxoviruses is still unclear. Understanding the interaction of N and M proteins would provide insights into the virus assembly and budding, thereby contributing to the development of new strategies for the inhibition of *Henipavirus* replication and also aid in the development of potential antivirals.

Ecsherichia coli has been used successfully to express NiV N protein which assembles into herringbone-like capsid structures, but it failed to resemble the native viral structures. The M protein was also produced using the *E. coli* expression system and similarly the VLPs were morphologically different from the native virus. This reveals that the prokaryotic expression system may not be suitable to produce VLPs due to the lack of post-translational modification systems, especially for the expression of a membrane-bound protein such as the M protein. The disparities between the recombinant proteins produced in a prokaryotic and an eukaryotic system, suggests that the N and M proteins produced in an eukaryotic expression system could assemble into VLPs resembling the native virus. Seeking an alternative for the expression system, in this study the methylotrophic yeast, *Pichia pastoris* was explored as an eukaryotic expression system to produce the N and M proteins of NiV. The simplicity of the genetic manipulations and the availability of post-translational modification are the advantages of *P. pastoris* expression system. The *P. pastoris* expression system is hypothesized to produce biologically active N and M proteins.

Thus the objectives of this study were:

1. To clone and express the coding sequences of nucleocapsid and matrix protein of Nipah virus in *Pichia pastoris*,
2. To purify the recombinant nucleocapsid and matrix proteins produced in *Pichia pastoris*,
3. To study the interaction of the nucleocapsid and matrix proteins of Nipah virus.

REFERENCES

Albani, J.R. 2007. Fluorescence quenching, In *Fluorescence Spectroscopy*, pp139-159. Oxford:Blackwell Publishing.

Andrusier, N., Nussinov, R. and Wolfson, H.J. 2007. FireDock: fast interaction refinement in molecular docking. *Proteins* 69(1):139-159.

Anfinsen, C.B.1973. Principles that govern the folding of protein chains. *Science* 181(4096): 223-230.

Arankalle,V.A., Bandyopadhyay,B.T., Ramdasi,A.Y., Jadi,R., Patil,D.R., Rahman, M., Majumdar, M., Banerjee, P.S., Hati, A.K., Goswami, R.P., Neogi, D.K. and Mishra, A.C. 2011. Genomic characterization of Nipah virus, West Bengal, India. *Emerging Infection Disease*s 17(5): 907-909.

Baharuddin, A., Amir, H.A., Othman, R., Xu, Y., Huang, M., Tejo, A., B., Yusof, R., Abdul Rahman, N., Othman, S. 2014. Dengue envelope domain III-peptide binding analysis via tryptophan fluorescence quenching assay. *Chem Pharm Bull (Tokyo)* 62(10):947-955.

Baneyx, F. and Mujacic, M. 2004. Recombinant protein folding and misfolding in *Escherichia coli. Nature Biotechnology* 22(11):1399-408.

Battisti, A.J., Meng, G., Winkler, D.C., McGinnes, L.W., Plevka, P., Steven, A.C., Morrison, T.G. and Rossmann, M.G. 2012**.** Structure and assembly of a paramyxovirus matrix protein. *Proceedings of the National Academy of Sciences of the United States of America* 109(35):13996-4000.

Bhella, D., Ralph, A. and Yeo, R.P. 2004. Conformational flexibility in recombinant measles virus nucleocapsids visualised by cryo-negative stain electron microscopy and real-space helical reconstruction. *Journal of Molecular Biology* 340:319-331.

Bian, Q., Liu, J., Tian, J. and Hu, Z. 2004. Binding of genistein to human serum albumin demonstrated using tryptophan fluorescence quenching. *International Journal of Biological Macromolecules* 34(5):275–279.

Bonaparte, M.I., Dimitrov, A.S., Bossart, K.N., Crameri, G., Mungall, B.A., Bishop, K.A., Choudhry, V., Dimitrov, D.S., Wang, L.F., Eaton, B.T. and Broder, C.C. 2005. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proceedings of the National Academy of Sciences of the United States of America* 102(30):10652–10657.

Bossart, K.N., Wang, L.F., Eaton, B.T. and Broder, CC. 2001. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. *Virology* 290(1):121–135.

Bossart, K.N., Crameri, G, Dimitrov, A.S., Mungall, B.A., Feng, Y.R., Patch, J.R., Choudhary, A., Wang, L.F., Eaton, B.T. and Broder, C.C. 2005. Receptor binding, fusion inhibition, and induction of crossreactive neutralizing

antibodies by a soluble G glycoprotein of Hendra virus. *Journal of Virology* 79(11): 6690-6702.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 17:248-254.

Bruhn, J. F., Barnett, K.C., Bibby, J., Thomas, J. M. H., Keegan, R.M., Rigden, D. J., Bornholdt, Z.A. and Saphire, E.O. 2014. Crystal Structure of the Nipah Virus Phosphoprotein Tetramerization Domain. *Journal of Virology* 88:758 – 762.

Bruhn-Johannsen, J., Barnett, K., Bibby, J., Thomas, J., Keegan, R., Rigden, D.J., Bornholdt, Z. and Saphire, E. 2014. Crystal structure of the Nipah virus phosphoprotein tetramerization domain. *Journal of Virology* 88:758-762.

Cereghino, J.L. and Cregg, J.M. 2000. Heterologous protein expression in the methylotrophic yeast *Pichia pastoris. FEMS Microbiology Reviews* 24(1):45-66.

Cereghino, G.P.L., Cereghinom J,L, Ilgen, C. and Cregg, J.M. 2002. Production of recombinant proteins in fermenter cultures of the yeast Pichia pastoris. *Current Opinion in Biotechnology* 13:329-332.

Chadha, M.S., Comer, J.A., Lowe, L., Rota, P.A., Rollin, P.E., Bellini, W.J., Ksiazek, T.G. and Mishra, A.C. 2006. Nipah Virus Associated Encephalitis Outbreak, Siliguri, India. *Emerging Infectious Diseases* 12 (2): 236-240.

Chan, Y.P., Chua, K.B., Lim, M.E. and Lam, S.K. 2001. Complete nucleotide sequences of Nipah virus isolates from Malaysia. *Journal of General Virology* 82:2151-2155.

Chan, Y.P., Koh, C.L., Lam, S.K. and Wang, L.F. 2004. Mapping of domains responsible for nucleocapsid protein–phosphoprotein interaction of Henipaviruses. *Journal of General Virology* 85:1675-1684.

Chen, J.M., Yu, M., Morrissy, C., Zhao, Y.G., Meehan, G., Sun, Y.X., Wang, Q.H., Zhang, W., Wang L.F. and Wang, Z.L. 2006. A comparative indirect ELISA for the detection of henipavirus antibodies based on a recombinant nucleocapsid protein expressed in *Escherichia coli. Journal of Virological Methods* 136:273-276.

Chen, R. 2012. Bacterial expression systems for recombinant protein production: *E. coli* and beyond. *Biotechnology Advances* 30(5): 1102-1107.

Chong, F.C., Tan, W.S., Biak, D.R.A., Ling, T.C. and Tey B.T. 2009a. Purification of histidine tagged nucleocapsid protein of Nipah virus using immobilized metal affinity chromatography. *Journal of Chromatography B* 877:1561-1567.

Chong, F.C., Tan, W.S., Biak, D.R.A., Ling, T.C. and Tey B.T. 2009b. Direct recovery of recombinant nucleocapsid protein of Nipah virus from unclarified *Escherichia coli* homogenate using hydrophobic interaction expanded bed

adsorption chromatography. *Journal of Chromatography A* 1217(8):1293-1297.

Chong, F.C., Tan, W.S., Biak, D.R.A., Ling, T.C. and Tey, B.T. 2010. Modulation of protease activity to enhance the recovery of recombinant nucleocapsid protein of Nipah virus. *Process Biochemistry* 45:133-137.

Chong, H.T., Hossain, M.J., Tan, C.T. 2008. Differences in epidemiologic and clinical features of Nipah virus encephalitis between the Malaysian and Bangladesh outbreaks. *Neurology Asia* 13: 23 – 26.

Chua, K.B., Goh, K.J., Wong, K.T., Kamarulzaman, A., Tan, P.S.K., Ksiazek, T.G., Zaki, S.R., Paul, G., Lam, S.K. and Tan CT. 1999 . Fatal encephalitis due to Nipah virus among pig farmers in Malaysia. *Lancet* 354:1257-1259.

Chua, K.B., Bellini, W.J., Rota, P.A., Harcourt, B.H., Tamin, A., Lam, S.K., Ksiazek, T.G., Rollin, P.E., Zaki, S.R., Shieh, W., Goldsmith, C.S., Gubler, D.J., Roehrig, J.T., Eaton, B., Gould, A.R., Olson, J., Field, H., Daniels, P., Ling, A.E., Peters, C.J., Andreson, L.J. and Mahy, B.W. 2000. Nipah Virus: A Recently Emergent Deadly Paramyxovirus. *Science* 288:1432-1435.

Chua, K.B., Koh, C.L., Hooi, P.S., Wee, K.F., Khong, J.H., Chua, B.H., Chan, Y.P., Lim, M.E. and Lam, S.K. 2002. Isolation of Nipah Virus from Malaysian Island Flying Foxes. *Microbes and Infection* 42:145-151.

Ciancanelli, M.J. and Basler, C.F. 2006. Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. *Journal of Virology* 80(24):12070-12078.

Ciancanelli, M.J., Volchkova, V.A., Shaw, M.L., Volchkov, V.E. and Basler, C.F. 2009. Nipah virus sequesters inactive STAT1 in the nucleus via a P gene-encoded mechanism. *Journal of Virology* 83:7828-7841.

Cockell, S.J., Oliva, B. and Jackson, R.M. 2007. Structure-based evaluation of in silico predictions of protein-protein interactions using comparative docking. *Bioinformatics* 23(5): 573-581.

Communie, G., Rob, W.H., Ruigrok, R.W.H., Jensen, M.R. and Blackledge, M. 2014. Intrinsically disordered proteins implicated in paramyxoviral replication machinery. *Current Opinion in Virology* 5:72-81.

Coronel, E.C., Murti, K.G., Takimoto, T. and Portner, A. 1999. Human parainfluenza virus type 1 matrix and nucleoprotein genes transiently expressed in mammalian cells induce the release of virus-like particles containing nucleocapsid-like structures. *Journal of Virology* 73:7035-7038.

Coronel, E.C., Takimoto, T., Murti, K.G., Varich, N. and Portner, A. 2001. Nucleocapsid incorporation into parainfluenza virus is regulated by specific interaction with matrix protein. *Journal of Virology* 75:1117-23.

Craghill, J., Cronshaw, A.D. and Harding, J.J. 2004. The identification of a reaction site of glutathione mixed-disulphide formation on γ S-crystalline in human lens. *Biochemistry Journal* 379:595-600.

Crameri, G., Wang, L.F., Morrissy, C., White, J. and Eaton, B.T. 2002. A rapid immune plaque assay for the detection of Hendra and Nipah viruses and anti-virus antibodies. Journal of *Virological Methods* 99:41-51.

Crameri, G., Todd, S., Grimley, S., McEachern, J.A., Marsh, G.A., Smith, C., Tachedjian, M., De Jong, C., Virtue, E.R., Yu, M., Bulach, D., Liu, J.P., Michalski, W.P., Middleton, D., Field, H.E. and Wang, L.F. 2009. Establishment, immortalisation and characterisation of pteropid bat cell lines. *PLoS One* 4:e8266.

Cregg, J.M., Barringer, K.J., Hessler, A.Y. and Madden, K.R. 1985. *Pichia pastoris* as a host system for transformations. *Molecular and Cellular Biology* 5(12):3376-3385.

Cregg, J.M, Madden, K.R., Barringer, K.J., Thill, G.P. and Stillman, C.A. 1989. Functional characterization of the two alcohol oxidase genes from the yeast *Pichia pastoris*. *Molecular and Cellular Biology* (3):1316-1323.

Cregg., J.M., Cereghino, J.L., Shi, J. and Higgins, D.R. 2000. Recombinant protein expression in *Pichia pastoris*. *Molecular Biotechnology* 16(1):23-52.

Daniels, P., Ksiazek, T. and Eaton, B.T. 2001. Laboratory diagnosis of Nipah and Hendra virus infections. *Microbes and Infections* 3:289-295.

Diederich, S., Moll, M., Klenk, H.D. and Maisner, A. 2005. The Nipah virus fusion protein is cleaved within the endosomal compartment. *The Journal of Biological Chemistry* 280:29899-29903.

Duhovny, D., Nussinov, R., and Wolfson, H.J. 2002. Efficient Unbound Docking of Rigid Molecules. In Proceedings of the Second International Workshop on Algorithms in Bioinformatics (Springer-Verlag), pp. 185-200.

Duhovny, D., Inbar, Y., Nussinov, R. and Wolfson, H.J. 2005. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Research* 33 (Web Server issue):W363-W367.

Dutch, R.E. 2010. Entry and Fusion of Emerging Paramyxoviruses. *PLoS Pathogens* 6(6) e1000881.

Eaton, B.T., Broder, C.C., Middleton, D. and Wang, L.F. 2006. Hendra and Nipah Viruses: different and dangerous. *Nature Reviews* 4:23-35.

Epstein, J.H., Prakash, V.B., Smith, C.S., Daszak, P., McLaughlin, A.B., Meehan, G., Field, H.E. and Cunningham, A.A. 2008. Henipavirus infection in Fruit Bats (Pteropus giganteus),India. *Emerging Infectious Diseases*14(8):1309-1311.

Eshaghi, M., Tan, W.S., Ong, S.T. and Yusoff, K. 2005. Purification and characterization of Nipah virus nucleocapsid protein produced in insect cells. *Journal of Clinical Microbiology* 43:3172-3177.

Fauquet, C.M., Mayo,M.A., Maniloff, J., Desselberger, U. and Ball, L.A. 2005. Virus taxonomy. Classification and nomenclature of viruses. Eighth Report of the ICTV, Elsevier, San Diego.

Field, H., Young, P., Yob, J.M., Mills, J., Hall, L. and Mackenzie, J. 2001.The natural history of Hendra and Nipah viruses. *Microbes Infect.* 3:307-314.

Fiser, A., Feig, M., Brooks, C.L. and Sali, A. 2002. Evolution and physics in comparative protein structure modeling. *Accounts of Chemical Research* 35:413.

Fiser, A. 2004. Protein structure modeling in the proteomics era. *Expert Review of Proteomics* 1(1):97-110.

Floudas, C.A., Fung, H.K., McAllister, S.R., Monnigmann, M. and Rajgaria, R. 2006. Advances in protein structure prediction and de novo protein design: A review. *Chemical Engineering Science* 61(3):966-988.

Freed, E.O. 2002. Viral late domains. *Jornal of Virology* 76:4679-4687

Gafvelin, G., Sakaguchi, M., Andersson, H. and Heijne, G.V. 1997. Topological rules for membrane protein assembly in eukaryotic cells. *Journal of Biolological Chemistry* 272:6119-6127.

Goh, K.J., Tan, C.T., Chew, N.K., Tan, P.S., Kamarulzaman, A., Sarji, S.A., Wong, K.T., Abdullah, B.J., Chua, K.B. and Lam, S.K. 2000.Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *The New England Journal of Medicine* 342:1229-1235.

Goldsmith, C.S., Whistler, T., Rollin, P.E., Ksiazek, T.G., Rota, P.A., Bellini, W.J., Daszak, P., Wong, K.T., Shieh, W.J., and Zaki, S.R. 2003. Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. *Virus Research* 92:89-98.

Gomez, P.P., Albo, C., Pastrana, E.P., Vivo, A. and Portela, A. 2000. Influenza virus matrix protein is the major driving force in virus budding. *Journal of Virology* 74:11538-11547.

Gorinstein, S., Goshev, I., Moncheva, S., Zemser, M., Weisz, M., Caspi, A., Libman, I., Lerner, H.T., Trakhtenberg, S. and Mart ń-Belloso, O. 2000. Intrinsic tryptophan fluorescence of human serum proteins and related conformational changes. *Journal of Protein Chemistry* 19(8):637-642.

Guillaume, V., Lefeuvre, A., Faure, C., Marianneau, P., Buckland, R., Lam, S.K., Wild, T.F. and Deubel, V. 2004. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). *Journal of Virological Methods* 120:229-237.

Guillaume, V., Aslan, H., Ainouze, M., Guerbois, M., Wild, T.F., Buckland, R. and Langedijk JP. 2006. Evidence of a potential receptor-binding site on the Nipah virus G protein (NiV-G): identification of globular head residues with a role in fusion promotion and their localization on an NiV-G structural model. *Journal of Virology* 80:7546-7554.

Gurramkonda, C., Zahid, M., Nemani, S.K., Adnan, A., Gudi, S.K., Khanna, N., Ebensen, T., Lünsdorf, H., Guzmán, C.A.and Rinas, U. 2013. Purification of hepatitis B surface antigen virus-like particles from recombinant *Pichia pastoris* and *in vivo* analysis of their immunogenic properties. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences* 940: 104-111.

Habchi, J., Blangy, S., Mamelli, L., Jensen, M.R., Blackledge, M., Darbon, H., Oglesbee, M., Shu, Y. and Longhi, S. 2011. Characterization of the interactions between the nucleoprotein and the phosphoprotein of Henipavirus. *Journal of Biological Chemistry* 286:13583-13602.

Habchi, J. and Longhi, S. 2012. Structural disorder within paramyxovirus nucleoproteins and phosphoproteins. *Molecular BioSystems* 8:69-81.

Halpin, K., Bankamp, B., Harcourt, B.H., Bellini, W.J. and Rota, P.A. 2004. Nipah virus conforms to the rule of six in a minigenome replication assay. *Journal of General Virology* 3:701-707.

Halpin, K. and Mungall, B.A. 2007. Recent progress in henipavirus research. *Comparative Immunology, Microbiology and Infectious Diseases* 30:287-307.

Harcourt, B. H., Tamin, A., Ksiazek, T. G., Rollin, P. E., Anderson, L. J., Bellini, W. J. & Rota, P. A. 2000. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology* 271: 334-349.

Hanna, M., Xiao, W. 2006. Isolation of Nucleic Acids. In *Yeast protocol: Methods in Molecular Biology*, ed. W. Xiao, pp 15-20. Totowa:Humana Press.

Harcourt BH, Tamin A, Halpin K, Ksiazek TG, Rollin PE, Bellini WJ, Rota PA. 2001. Molecular characterization of the polymerase gene and genomic termini of Nipah virus. *Virology* 287(1):192-201.

Harcourt, B.H. 2005. Genetic characterization of nipah virus, Bangladesh, 2004. *Emerging Infectious Diseases* 11: 1594-1597.

Harouaka, D. and Wertz, G.W**.** 2009. Mutations in the C-terminal loop of the nucleocapsid protein affect vesicular stomatitis virus RNA replication and transcription differentialy. *Journal of Virology* 83**:**11429-11439.

Harrison, M.S., Sakaguchi, T. and Schmitt, A.P. 2010. Paramyxovirus Assembly and Budding: Building Particles that Transmit Infections. *The International Journal of Biochemistry and Cell Biology* 42(9): 1416-1429.

Hasslacher, M., Schall, M., Hayn, M., Bona, R., Rumbold, K., Lückl, J., Griengl, H., Kohlwein, S.D. and Schwab, H. 1997. High-level intracellular expression of hydroxynitrile lyase from the tropical rubber tree Hevea brasiliensisin microbial hosts. *Protein Expresion and Purification* 11(1):61-71.

Hausmann, S., Jacques, J.P. and Kolakofsky, D. 1996. Paramyxovirus RNA editing and the requirement for hexamer genome length. *RNA* 2(10):1033–1045.

Helles, G. 2008. A comparative study of the reported performance of ab initio protein structure prediction algorithms. *Journal of the Royal Society Interface* 5(21):387-396.

Ho, J.D., Yeh, R., Sandstrom, A., Chorny, I., Harries, W.E.C, Robbins, R.A., Larry, J., Miercke, W. and Stroud, R.M. 2009. Crystal structure of human aquaporin 4 at 1.8 Å and its mechanism of conductance. *Proceedings of the National Academy of Sciences of the United States of America* 106(18): 7437-7442.

Hosur, R., Peng, J., Vinayagam, A., Stelzl, U., Xu, J., Perrimon, N., Bienkowska, J. and Berger, B. 2012. "A computational framework for boosting confidence in high-throughput protein-protein interaction datasets," *Genome Biology*, 13(8):R76.

Huang, M., Sato, H., Hagiwara, K., Watanabe, A., Sugaim, A., Ikeda, F., Kozuka-Hata, H., Oyama, M., Yoneda, M. and Kai, C. 2011. Determination of a phosphorylation site in Nipah virus nucleoprotein and its involvement in virus transcription. *Journal of General Virology* 92:2133-2141.

Hyatt, A.D., Zaki, S.R., Goldsmith, C.S., Wise, T.G. and Hengstberger, S.G. 2001. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes and Infections* 3:297-306.

Iehlé, C., Razafitrimo, G., Razainirina, J., Goodman, S.M., Faure, C., Georges-Courbot, M.C., Rousset, D., Reynes, J.M. and Andriaholinirina, N. 2007. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. *Emerging Infectious Diseases* 13(1):159-161.

Inoue, M., Tokusumi, Y., Ban, H., Kanaya, T., Shirakura, M., Tokusumi, T., Hirata, T., Nagai, Y., Iida, A. and Hasegawa, M. 2003. A new Sendai virus vector deficient in the matrix gene does not form virus particles and shows extensive cell-to-cell spreading. *Journal of Virology* 77: 6419-6429.

Iwasaki, M., Takeda, M., Shirogane, Y., Nakatsu, Y., Nakamura, T. and Yanagi, Y. 2009. The matrix protein of measles virus regulates viral RNA synthesis and assembly by interacting with the nucleocapsid protein. *Journal of Virology* 83:10374-10383.

Johnson, M.S., Srinivasan, N., Sowdhamini, R. and Blundell, T.L. 1994. Knowledge-based protein modeling. *Critical Reviews in Biochemistry and Molecular Biology* 29:1.

Juozapaitis, M., Slibinskas, R., Staniulis, J., Sakaguchi, T. and Sasnauskas, K. 2005. Generation of Sendai virus nucleocapsid-like particles in yeast. *Virus Research* 108:221-224.

Justice, P.A., Sun, W., Li, Y., Ye, Z., Grigera, P.R. and Wagner, R.R. 1995. Membrane vesiculation function and exocytosis of wild type and mutant matrix proteins of vesicular stomatitis virus. *Journal of Virology* 69:3156-3160.

Kaku, Y., Noguchi, A., Marsh, G.A., McEachern, J.A., Okutani, A., Hotta, K., Bazartseren, B., Fukushi, S., Broder, C.C., Yamada, A., Inoue, S. and Wang, L.F. 2009. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *Journal of Virological Methods* 160:7-13.

Kaku, Y., Noguchi, A., Marsh, G.A., Barr, J.A., Okutani, A., Hotta, K., Bazartseren, B., Fukushi, S., Broder, C.C., Yamada, A., Inoue, S. and Wang, L.F. 2012. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *Journal of Virological Methods* 179:226-232.

Karplus, K., Barrett, C. and Hughey, R. 1998. Hidden Markov models for detecting remote protein homologies. *Bioinformatics* 14:846–856.

Kashiwazaki, Y., Na, Y.N., Tanimura, N. and Imada, T. 2004. A solid-phase blocking ELISA for detection of antibodies to Nipah virus. *Journal of Virological Methods* 121(2):259-61.

Kemege, K.E., Hickey, J.M., Lovell, S. and Battaile, K.P. 2011. *Ab Initio* Structural Modeling of and Experimental Validation for *Chlamydia trachomatis* Protein CT296 Reveal Structural Similarity to Fe(II) 2-Oxoglutarate-Dependent Enzymes. *Journal of Bacteriology* 193(23): 6517-6528.

Kho, C.L., Tan, W.S., Tey, B.T. and Yusoff, K. 2003. Newcastle disease virus nucleocapsid protein: self-assembly and length-determination domains. *Journal of General Virology* 84:2163-8.

Ksiazek TG, Rota PA, Rollin PE (2011) A review of Nipah and Hendra viruses with an historical aside. *Virus Research* 162 (1–2):173-183.

Laemmli, UK. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227:680-685.

Lakowicz J. R. 2006. Principles of fluorescence spectroscopy, Kluwer Academic/Plenum Publishers, New York, pp. 1-24.

Lakowicz J. R. 2006. Principles of fluorescence spectroscopy, Kluwer Academic/Plenum Publishers, New York, pp. 278-327.

Lam, S.K., Chua, K.B. 2002. Nipah Virus encephalitis outbreak in Malaysia. *Clinical Infectious Diseases* 34 (supplement 2): S48-S51.

Lamb, R. A. and Kolakofsky, D. 2001. Paramyxoviridae: the viruses and their replication. In *Fields Virology*, Edited by Knipe, D.M., Howley, P.M. and Griffin, D.E. pp. 1305–1340, Philadelphia: Lippincott Williams and Wilkins.

Lamb, R.A. and Parks, G.D. 2007. Paramyxoviridae: the viruses and their replication. In *Fields virology* Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin, M.A., Roizman, B. and Straus, S.E., 5th ed. pp 1449-1496. Lippincott – Raven Publishers Philadelphia, PA.

Lee, J., Wu, S. and Zhang, Y. 2009. *Ab Initio* Protein Structure Prediction, *From Protein Structure to Function with Bioinformatics*, Springer Science, UK, pp 3-25.

Li, M., Schmitt, P.T., Li, Z., McCrory, T.S., He, B. and Schmitt, A.P. 2009. Mumps virus matrix, fusion, and nucleocapsid proteins cooperate for efficient production of virus-like particles. *Journal of Virology* 83(14):7261-7272.

Licata, J. M., Johnson, R. F., Han, Z. and Harty, R. N. 2004. Contribution of Ebola virus glycoprotein, nucleoprotein, and VP24 to budding of VP40 virus-like particles. *Journal of Virology* 78:7344–7351.

Lo, M.K. and Rota, P.A. 2008.The emergence of Nipah virus, a highly pathogenic paramyxovirus. *Journal of Clinical Virology* 43:396-400

Lo, M.K., Lowe, L., Hummel, K.B., Sazzad, H.M.S., Gurley, E.S., Hossain, M.J., Luby, S.P., Miller, D.B., Comer, J.A., Rollin, P.E., Bellini, W.J. and Rota, P.A. 2012. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. 2012. [Electronic version]. *Emerging Infectious Diseases* 18:2.

Lu, H. and Skolnick, J. 2001. A distance-dependent atomic knowledge-based potential for improved protein structure selection. *Proteins* 44(3):223-232.

Luby, S.P., Gurley, E.S. and Hossain, M.J. 2009. Transmission of human infection with Nipah Virus. *Clinical Infectious Diseases* 49 (11):1743-1748.

Lünsdorf, H., Gurramkonda, C., Adnan, A., Khanna, N. and Rinas, U. 2011. Virus-like particle production with yeast: ultrastructural and immunocytochemical insights into *Pichia pastoris* producing high levels of the hepatitis B surface antigen. *Microbial Cell Factories* 10:48-58.

Magoffin, D.E., Halpin, K., Rota, P.A. and Wang, L.F. 2007. Effects of single amino acid substitutions at the E residue in the conserved GDNE motif of the Nipah virus polymerase (L) protein. *Archives of Virology* 152(4):827-832.

Mashiach, E., Schneidman-Duhovny, D., Andrusier, N., Nussinov, R. and Wolfson, H.J. 2008. FireDock: a web server for fast interaction refinement in molecular docking *Nucleic Acids Research* (36):229-232.

Meulendyke, K.A., Wurth, M.A., McCann, R.O. and Dutch, R.E. 2005. Endocytosis plays a critical role in proteolytic processing of the Hendra virus fusion protein. *Journal of Virology* 79 (20):12643-12649.

Moeller, A., Kirchdoerfer, R.N., Potter, C. S., Carragher, B., Wilson, I.A. 2012. Organization of the Influenza Virus Replication Machinery. *Science* 21(338): 1631-1634.

Mohd Nor, M.N., Gan, C.H. and Ong, B.L. 2000. Nipah virus infection of pigs in peninsular Malaysia. *Revue Scientifique Et Technique* 19:160-165.

Möller, M. and Denicola, A. 2002. Protein tryptophan accessibility studied by fluorescence quenching. *Biochemistry And Molecular Biology Education* 30:175-178.

Montgomery, J.M., Hossain, M.J., Gurley, E.S., Carroll, D.S. Croisier, A., Bertherat, E., Asgari, N., Formenty, P., Keeler, N., Comer, Bell, J.M.R., Akram, K., Molla, A.R., Zaman, K., Islam, M.R., Wagoner, K., Mills, J.N., Rollin, P.E., Ksiazek, T.G. and Breiman, R.F. 2008. Risk Factors for Nipah Virus Encephalitis in Bangladesh. *Emerging Infectious Diseases* 10:1526-1532.

Morin, B., Kranzusch, P.J., Rahmeh, A.A. and Whelan, S.P. 2013. The polymerase of negative-stranded RNA viruses. *Current Opinion in Virology* 3:103-110.

Mountcastle, W.E., Compans, R.W., Lackland, H. and Purnell, W.C. P. 1974 Proteolytic cleavage of subunits of the nucleocapsid of the paramyxovirus Simian virus 5. *Journal of Virology* 14:1253-1261.

Najjar, F., Schmitt, AP. and Dutch, R.E. 2014. Paramyxovirus glycoprotein incorporation, assembly and budding: a three way dance for infectious particle production. *Viruses* 6(8):3019-3054.

Nayak, D., Panda, D., Das, S.C., Luo, M. and Pattnaik, A.K. 2009. Singleamino-acid alterations in a highly conserved central region of vesicular stomatitis virus N protein differentially affect the viral nucleocapsid template functions. Journal of Virology 83:5525-5534.

Negrete, O.A., Wolf, M.C., Aguilar, H.C., Enterlein, S., Wang, W., Mühlberger, E., Su, S.V., Bertolotti-Ciarlet, A., Flick, R. and Lee, B. 2006. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathogens* 2:e7.

OIE Terrestrial Manual. 2011. Hendra and Nipah virus diseases. In *Manual of diagnostic tests and vaccines for terrestrial animals*. OIE, Paris.

Omi-Furutani, M., Yoneda, M., Fujita, K., Ikeda, F. and Kai, C. 2010. Novel phosphoprotein-interacting region in Nipah virus nucleocapsid protein and its involvement in viral replication. *Journal of Virology* 84:9793-9799.

Ong, S.T., Yusoff, K., Kho, C.L., Abdullah, J.O. and Tan, W.S. 2009. Mutagenesis of the nucleocapsid protein of Nipah virus involved in capsid assembly. *Journal of General Virology* 90:392-397.

Ozimek, P., Veenhuis, M., and van der Klei, I. J. 2005. Alcohol oxidase: A complex perixisomal, oligomeric flavoprotein. *FEMS Yeast Research* 5:975-983.

Parashar , U.D., Sunn, L.M., Ong, F., Mounts, A.W., Arif, M.T., Ksiazek, T.G., Kamaluddin, M.A., Mustafa, A.N., Kaur, H., Ding, L.M., Othman, G.,Radzi, H.M., Kitsutani, P.T., Stockton, P.C., Arokiasamy, J., Gary, H.E.J. and Anderson, L.J. 2000. Case-control study of risk factors for human infection with the new zoonotic paramyxovirus, Nipah virus, during a 1998–1999 outbreak of severe encephalitis in Malaysia. *Journal of Infectious Diseases* 181:1755-1759.

Patch, J.R., Crameri, G., Wang, L.F., Eaton, B.T. and Broder, C.C. 2007. Quantitative analysis of Nipah virus proteins released as virus-like particles reveals central role for the matrix protein. *Virology Journal* 4(4):1-14.

Patch, J.R., Han, Z., McCarthy, S.E., Yan, L., Wang, L.F., Harty, R.N. and Broder, C.C. 2008. The YPLGVG sequence of the Nipah virus matrix protein is required for budding. *Virology Journal* 5(137):1-12.

Patrick, S.M., Fazenda, M.L., McNeil, B. and Harvey, L.M. 2005. Heterologous protein production using the *Pichia pastoris* system. *Yeast* 22:249-70.

Pernet, O., Pohl, C., Ainouze, M., Kweder, H. and Buckland, R. 2009. Nipah virus entry can occur by macropinocytosis. *Virology* 395(2):298–311.

Phizicky, E.M. and Fields, S. 1995. Protein-Protein Interactions: Methods for Detection and Analysis. *Microbiological Reviews* (59) :94-123.

Pieper, U., Eswar, N., Braberg, H., Madhusudhan, M.S., Davis, F.P., Stuart, A.C., Mirkovic, N., Rossi, A., Marti-Renom, M.A., Fiser, A., Webb, B., Greenblatt, D., Huang, C.C., Ferrin, T.E. and Sali, A. 2006. MODBASE: a database of annotated comparative protein structure models and associated resources. *Nucleic Acids Research* (34): 291-295.

Rawel, H.M., Frey, S.K., Meidtner, K., Kroll, J. and Schweigert, F.J. 2006. Determining the binding affinities of phenolic compounds to proteins by quenching of the intrinsic tryptophan fluorescence. *Molecular Nutrition and Food Research* 50(8):705-713.

Reynes, J.M., Counor, D., Ong, S., Faure, C., Seng, V., Molia, S., Walston, J., Georges-Courbot, M.C., Deubel, V. and Sarthou, J.L. 2005. Nipah virus in Lyle's flying foxes, Cambodia. *Emerging Infectious Diseases* 11(7):1042-1047.

Rosano, G.L. and Ceccarelli, E.A. 2014. Recombinant protein expression in *Escherichia coli*: advances and challenges. *Frontiers in Microbiology* 5(172):1-17.

Roy, A., Kucukural, A. and Zhang, Y. 2010. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols* 5(4): 725-738.

Ruigrok, R.W., Cre´ pin, T. and Kolakofsky, D. 2011. Nucleoproteins and nucleocapsids of negative-strand RNA viruses. *Current Opinion in Microbiology* 14:504-510.

Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. *Molecular cloning: A laboratory manual.* Laboratory 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York**.**

Sanderson, C.M., Wu, H.H. and Nayak, D.P. 1994. Sendai virus M protein binds independently to either the F or the HN glycoprotein in vivo. *Journal of Virology* 68:69-76.

Schmitt, A. P., Leser, G.P., Waning, D.L. and Lamb, R.A. 2002. Requirements for budding of paramyxovirus simian virus 5 virus-like particles. *Journal of Virology* 76:3952-3964.

Schmitt, A.P. and Lamb, R.A. 2004. Escaping from the cell: assembly and budding of negative-strand RNA viruses. *Current Topics in Microbiology and Immunology* 283:145-196.

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R. and Wolfson, H.J. 2005. PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Research* 33:W363-367.

Shahandeh, A. and Rabu, A. B. 2013. Functional analysis of the L Protein of Nipah Virus Using Minigenome System. *International Journal of Fundamental Physical Sciences* 3(3): 36-42.

Shatsky, M., Dror, O., Schneidman-Duhovny, D., Nussinov, R. and Wolfson, H.J. 2004. BioInfo3D: a suite of tools for structural bioinformatics. [Electronic version]. *Nucleic Acids Research* 1(32):W503.

Shen, M.Y. and Sali, A. 2006. Statistical potential for assessment and prediction of protein structures. *Protein Science* 15(11):2507-2524.

Sigoillot, M., Brockhoff, A., Lescop, E., Poirier, N., Meyerhof, W. and Briand, L. 2012. Optimization of the production of gurmarin, a sweet-taste-suppressing protein, secreted by the methylotrophic yeast *Pichia pastoris*. *Applied Microbiology and Biotechnology* (96):1253-1263.

Sipos, L. and Gunnar, V. H. 1993. Predicting the topology of eukaryotic membrane proteins. *European Journal of Biochemistry* 213:1333-1340.

Skolnick, J., Jaroszewski, L., Kolinski, A. and Godzik, A. 1997. Derivation and testing of pair potentials for protein folding. When is the quasichemical approximation correct? *Protein Science* 6:676-688.

Skolnick, J. 2006. In quest of an empirical potential for protein structure prediction. *Current Opinion in Structural Biology* 16(2):166-171.

Sleeman, K., Bankamp, B., Hummel, K.B., Lo, M.K., Bellini, W.J. and Rota, P.A. 2008. The C, V and W proteins of Nipah virus inhibit minigenome replication. *Journal of General Virology* 89:1300-1308.

Slibinskas, R., Samuel, D., Gedvilaite, A., Staniulis, J. and Sasnauskas, K. 2004. Synthesis of the measles virus nucleoprotein in yeast *Pichia pastoris* and *Saccharomyces cerevisiae*. *Journal of Biotechnology* 107:115-24.

Sørensen, H.P. and Mortensen, K. K. 2005. Soluble expression of recombinant proteins in the cytoplasm of *Escherichia coli*. *Microbial Cell Factories* 4(1):1-8.

Sousa, S.F., Fernandes, P.A. and Ramos, M.J. 2006. Protein-ligand docking: current status and future challenges. *Proteins* 65(1):15-26.

Subramaniam, S.K., Tey, B.T., Hamid, M. and Tan, W.S. 2009. Production of the matrix protein of Nipah virus in *Escherichia coli*. *Journal of Virological Methods* 162:179-183.

Sugahara, F., Uchiyama, T., Watanabe, H., Shimazu, Y., Kuwayama, M., Fujii, Y., Kiyotami, K., Adachi, A., Kohno, N., Yoshida, T. and Sakaguchi, T. 2004. Paramyxovirus Sendai virus-like particle formation by expression of multiple viral proteins and acceleration of its release by C protein. *Journal of Virology* 325:1-10.

Takimoto, T., Bousse, T., Coronel, E.C., Scroggs, R.A. and Portner, A. 1998. Cytoplasmic domain of Sendai virus HN protein contains a specific sequence required for its incorporation into virions. *Journal of Virology* 72**:**9747-9754.

Takimoto, T., Murti, K.G., Bausse, T., Scroggs, R. and Portner, A. 2001. Role of matrix and fusion proteins in budding of Sendai virus. *Journal of Virology* 75:11384-11391.

Takimoto, T., and Portner, A. 2004. Molecular mechanism of paramyxovirus budding. *Virus Research* 106**:**133-145.

Tamin, A., Harcourt, B.H., Ksiazek, T.G., Rollin, P.E., Bellini, W.J. and Rota, P.A. 2002. Functional properties of the fusion and attachment glycoproteins of Nipah virus. *Virology* 296(1):190-200.

Tamin, A., Harcourt, B.H., Lo, M.K., Roth, J.A., Wolf, M.C., Lee, B., Weingartl, H., Audonnet, J.C., Bellini, W.J. and Rota, P.A. 2009. Development of a neutralization assay for Nipah virus using pseudotype particles. *Journal of Virological Methods* 160:1-6.

Tan, W.S., Ong, S.T., Eshaghi, M., Foo, S.S. and Yusoff, K. 2004. Solubility, immunogenicity and physical properties of the nucleocapsid protein of Nipah virus produced in *Escherichia coli. Journal of Medical Virology* 73:105-112.

Tarbouriech, N., Curran, J., Ebel, C., Ruigrok, R.W.H. and Burmeister, W.P. 2000. On the domain structure and the polymerization state of the Sendai virus P protein. *Virology* 266:99-109.

Tashiro, M., Seto, J.T., Klenk, H.D. and Rott, R. 1993. Possible involvement of microtubule disruption in bipolar budding of a Sendai virus mutant, F1-R, in epithelial MDCK cells. *Journal of Virology* 67:5902-5910.

Tashiro M, McQueen NL, Seto JT, Klenk HD and Rott R (1996) Involvement of the mutated M protein in altered budding polarity of a pantropic mutant, F1-R, of Sendai virus. *Journal of Virology* 70(9):5990-5997.

Tate, C.G. 2001. Overexpression of mammalian integral membrane proteins for structural studies. *FEBS Letters* 504:94-98.

Tawar, R.G., Duquerroy, S., Vonrhein, C., Varela, P.F., DamierPiolle, L., Castagne, N., MacLellan, K., Bedouelle, H., Bricogne, G., Bhella D., Eléouët, J.F. and Rey, F.A. 2009. Crystal structure of a nucleocapsid-like nucleoprotein-RNA complex of respiratory syncytial virus. *Science* 326:1279-1283.

Uppal, P.K. 2000. Emergence of Nipah virus in Malaysia. *Annals of the New York Academy of Sciences* 916:354-357.

Vivian, J.T. and Callis, P.R. 2001. Mechanisms of Tryptophan Fluorescence Shifts in Proteins. *Biophysical Journal* (80):2093-109.

Wacharapluesadee, S., Lumlertdacha, B., Boongird, K., Wanghongsa, S., Chanhome, L., Rollin, P., Stockton, P., Rupprecht, C.E., Ksiazek, T.G. and Hemachudha, T. 2005. Bat Nipah virus, Thailand. *Emerging Infectious Diseases* 11(12):1949-1951.

Walsh, G. 2010. Biopharmaceutical benchmarks 2010. *Nature Biotechnology* (9):917-24.

Wang, L.F., Harcourt, B.H., Yu, M., Tamin, A., Rota, P.A., Bellini, W.J. and Eaton, B.T. 2001. Molecular biology of Hendra and Nipah viruses. *Microbes and Infections* 3(4) :279-287.

Wang, Y.E., Park, A., Lake, M., Pentecost, M., Torres, B., Yun, TE., Wolf, M.C., Holbrook, M.R., Freiberg, A.N. and Lee, B. 2010. Ubiquitin-regulated nuclear-cytoplasmic trafficking of the Nipah virus matrix protein is important for viral budding. *PLoS Pathogens* 6(11):e1001186.

Wu, S. and Zhang, Y. 2007. LOMETS: a local meta-threading-server for protein structure prediction. *Nucleic Acids Research* 35:3375-3382.

Wu, S. and Zhang, Y. 2008. MUSTER: improving protein sequence profile-profile alignments by using multiple sources of structure information. *Proteins* 72(2):547-556.

Yin, J., Li, G., Ren, X. and Herrler, G. 2007. Select what you need: a comparative evaluation of the advantages and limitations of frequently used expression systems for foreign genes. *Journal of Biotechnology* 127(3):335-47.

Yob, J.M., Field, H., Rashdi, A.M., Morrissy, C., van der Heide, B., Rota, P., bin Adzhar, A., White, J., Daniels, P., Jamaluddin, A. and Ksiazek, T. 2001. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerging Infectious Diseases* 7(3):439-441.

Yu, F., Khairullah, N.S., Inoue, S., Balasubramaniam, V., Berendam, S.J., The, L.K., Ibrahim, N.S., Abdul Rahman, S., Hassan, S.S., Hasebe, F., Sinniah, M. and Morita, K. 2006. serodiagnosis using recombinant nipah virus nucleocapsid protein expressed in *Escherichia coli*. *Journal of Clinical Microbiology* 44:3134-3138.

Yu, X.W., Sha, C., Guo, Y.L., Xiao, R. and Xu, Y. 2013. High-level expression and characterization of achimeric lipase from *Rhizopus oryzae* for biodiesel production. *Biotechnology and Biofuels* (6):29.

Yusoff, K.and Tan, W.S. 2011. Newcastle disease virus: macromolecules and opportunities, *Avian Pathology* 30:439-455.

Zhang, Y., Kolinski, A. and Skolnick, J. 2003. TOUCHSTONE II: a new approach to *ab initio* protein structure prediction. *Biophysical Journal* 85(2):1145-1164.

Zhang, Y. and Skolnick, J. 2005. TM-align: a protein structure alignment algorithm based on the TMscore. *Nucleic Acids Research* 33:2302-2309.

Zhang, Y., Hubner, I.A., Arakaki, A.K., Shakhnovich, E. and Skolnick, J. 2006. *Proceedings of the National Academy of Sciences of the United States of America* 103:2605-2610.

Zhang Y. 2007. Template-based modeling and free modeling by I-TASSER in CASP7. *Proteins* 69:108-117.

Zhang, Y. 2008. I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 9(40):1-8.

Zhang, Q.C., Petrey, D., Deng, L., Qiang, L., Shi, Y., Thu, A.T., Bisikirska, B., Lefebvre, C., Accili, D., Hunter, T., Maniatis, T., Califano, A. and Honig, B. 2012. Structure-based prediction of protein-protein interactions on a genome-wide scale. *Nature*, 490(7421):556-560.

Zupan, A.L., Trobec, S., Gaberc-Porekar. V. and Menart, V. 2004. High expression of green fluorescent protein in *Pichia pastoris* leads to formation of fluorescent particles. *Journal of Biotechnology* 109:115-22.