Assembly of Arena virus an Ultra structural Perspective

Author's Details:

[(1)] **Dr. Abdullah Sethar**,D.V.M., M. Sc (Honors) from Pakistan and Ph.D from England, UK.Deputy Project Director,Sindh Agricultural Growth Project (Livestock Component) World Bank Assisted, Government of Sindh [(2)]**Dr. Benjamin W. Neuman**-School of Biological University of Reading-UK

[(3)]**Dr.Muhammad Ali Bhatti**-Assistant Professor-Agri:Business-IBA-Sukkur

Abstract: *The Arenaviredae are a family of viruses whose members are generally associated with rodent transmitted disease in humans which currently comprises 24 viral species. Arenavirus infections are relatively common in humans in some areas of the world and can cause severe illnesses including several haemorrhagic fevers. The virus particles vary in diameter from 60 to more than 300 nm. They are spherical and have a reported average diameter of 92 nanometres. All are enveloped in a lipid bilayer and have a bisegmented ambisense RNA genome, but relatively little is known about how virions are assembled and how virion structure relates to transmissibility. To investigate the role of each viral structural protein in forming and maintaining the structure of the virion, we have imaged particles of arenaviruses LCMV, PICV and TCRV, and compared their shape and structural characteristics to similar sized phospholipid vesicles. A very strong association between particle size and shape was found for all arenavirus particles: small virions were significantly rounder than vesicles of similar size, while large particles tended to be more elliptical in appearance. The natural variation in surface glycoprotein decoration and ribonucleoprotein incorporation was then measured. From this data it was concluded that there is no strong evidence relating particles size to decoration for arenaviruses as a group, but we did detect significant correlations between internal density and virion shape. Overall, we are able to conclude that small virions are round and relatively rigid compared to vesicles of the same size, while large virions are not. By comparing relative density of the membrane- proximal region it was discovered that arenavirus shape is controlled by complexes containing GPC, Z and NP at the surface of the virion, and that an unbroken inner shell of NP is essential for maintaining a rigid spherical shape. Furthermore, it was revealed that the inner leaflet of intact arenaviruses has a lower density than the inner leaflet of vesicles consistent with the interpretation that viral proteins are displaying lipid molecules from the inner leaflet of the viral membrane.These data provide a new way of assessing the function of viral protein interactions on virion structure and may be of use in designing antiviral drugs that act at the level of virion structure.*

Key words: *Arena virus, Ultra structural Perspective*

Introduction: 1.1 Molecular Biology of Arenaviruses:

The genus arenavirus belongs to family Arenaviridae [1]. This family currently comprises 24 viral species as recognized by the International Committee for Taxonomy of viruses [1].

Newly discovered arena-like viruses such as Kadoka, Morogoro, Pinhal, Chapare, Skinner Tank, Catarina and Dandenong virus, whose taxonomic status has not yet been determined by ICTV, represent putative new species [1]. Arenavirus can be divided into two serogroups which differ genetically and by geographical distribution [1]. It is assumed that humans usually become infected with arenavirus by inhalation of virus in aerosolized droplets of secretions or excretions from infected rodents [1]. The family Arenaviredae is divided into old world and new world complexes [2]. The New World serogroups can be subdivided into three clades, as shown in Fig. 1.1 [2]. All known arenaviruses are rodent-borne with the exception of Tacaribe virus, which was isolated from bats [3]. Host species for arenaviruses are listed in Table 1.1[2].

Table 1.1 List of arenavirus species and newly discovered arenavirus not yet classified, and respective characteristics (adapted from [2])

Virus	Acronym	Lineage	Country	Host	Path[5]
Allpahuayo	ALLV	NW-A[1]	Peru	*Oecomys bicolor, O. paricola*	
Amapari	AMAV	NW-B	Brazil	*Oryzomys goeldi, Neacomys guianae*	
Bear Canyon	BCNV	NW-Rec[2]	USA	*Peromyscus californicus, Neotoma macrotis*	
Catarina	NA[3]	NW-Rec	USA	*Neotoma micropus*	
Chapare	NA	NW-B	Bolvia	Unknown	Y
Cupixi	CPXV	NW-B	Brazil	*Oryzomys capito*	
Dandenong	NA	OW	Australia	Unknown	Y
Flexal	FLEV	NW-A	Brazil	*Oryzomys spp.*	
Guanarito	GTOV	NW-B	Venezuela	*Zygomtomys brevicauda, Sigmodon alstoni*	Y
Ippy	IPPYV	OW	Central African Republic	*Arvicanthus spp.*	
Junin	JUNV	NW-B	Argentina	*Callomys musculinus*	Y
Kodoko	NA	OW	Guinea	*Mus Nannomys minutoides*	
Lassa	LASV	OW	West Africa	*Mastomys natalensis*	Y
Latino	LATV	NW-C	Bolivia	*Callomys callosus*	
LCMV[4]	LCMV	OW	Ubiquitous	*Mus musculus, M. Domesticus*	Y
Lujo	LUJOV	OW	South Africa	Unknown	Y
Machupo	MACV	NW-B	Bolivia	*Callomys callosus*	Y
Mobala	MOBV	OW	Central African Republic	*Praomys spp.*	
Mopeia	MOPV	OW	Mozambique, Zimbabwe	*Mastomys natalensis*	
Morogoro	NA	OW	Tanzania	*Mastomys sp.*	
Oliveros	OLVV	NW-C	Argentina	*Bolomys spp.*	
Pampa	NA	NW-C	Argentina	*Bolomys spp.*	
Parana	PARV	NW-A	Paraguay	*Oryzomys buccinatus*	
Pichinde	PICV	NW-A	Colombia	*Oryzomys albigularis*	
Pinhal	NA	NW-C	Brazil	*Calomys tener*	
Pirital	PIRV	NW-A	Venezuela	*Sigmodon alstoni*	
Sabia	SABV	NW-B	Brazil	Unknown	Y
Skinner Tank	NA	NW-Rec	USA	*Neotoma Mexicana*	
Tonto Creek	NA	NW-Rec	USA	*Neotoma spp.*	
Tacaribe	TCRV	NW-B	Trinidad	*Artibeus bat*	
Tamiami	TAMV	NW-Rec	USA	*Sigmodon hispidus*	
Whitewater Arroyo	WWAV	NW-Rec	USA	*Neotoma spp.*	?

[1]New World (NW) virus clades A-C and Old World arenaviruses
[2]Recent New World arenavirus isolate
[3]Not available
[4]Lymphocytic Choriomeningitis Virus
[5]Significant human pathogens are marked "Y"

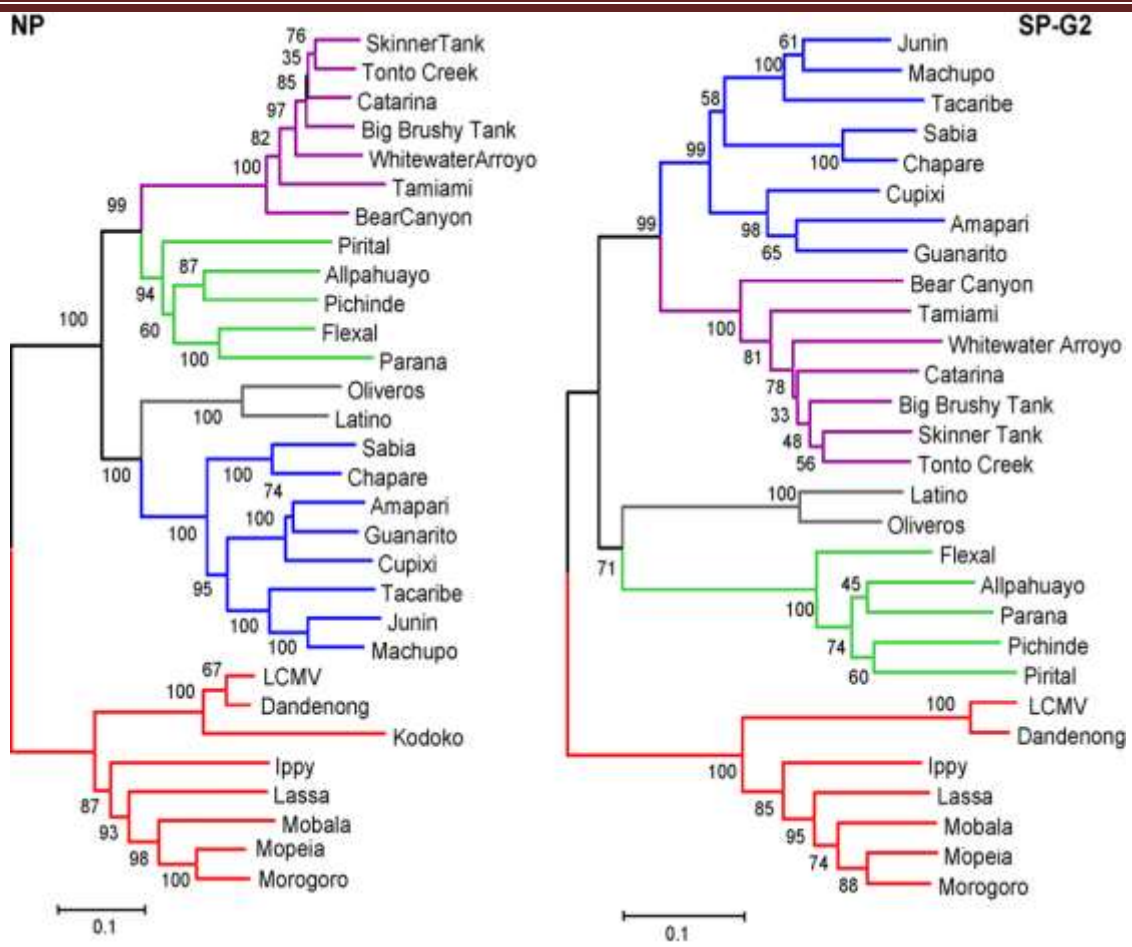


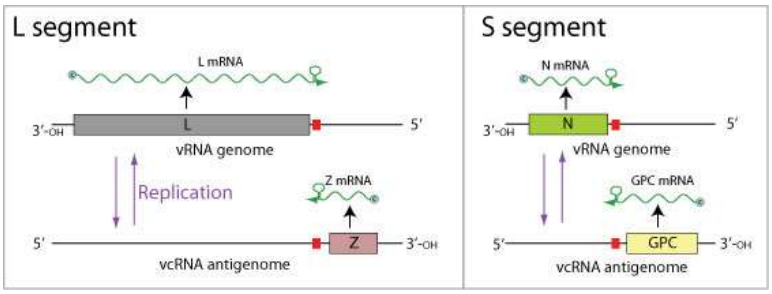
Figure 1.1 Phylogenetic relationships between arenaviruses within evolutionary lineages from [2]. Red, Old World viruses; Green, lineage A New World viruses, Blue, lineage B New World viruses; Grey, lineage C New World viruses; Purple, recombinant New World viruses. Left panel, phylogram based on nucleoprotein amino acid sequences, right panel, and phylogram based on concatenated signal peptide and glycoprotein two amino acid sequences [2].

Arenavirus particles are spherical and have an average diameter of 110-130nm [4]. All are enveloped in a lipid bilayer. Arenaviruses are enveloped and have a bisegmented ambisense RNA genome which is replicated in the same way as negative stranded RNA virus [4]. Each RNA segment has an ambisense coding strategy, encoding two proteins in opposite orientations, separated by an antigenic region [5-7]. The two genomic RNA segments are designated L and S and have approximate sizes of 7.2 and 3.4 kb, respectively [4, 8, 9].

All *cis*-acting signals required for encapsidation and polymerase entry of negative-strand RNA viruses appeared to be located within the 5` and 3` terminal untranslated regions (UTRs) [10]. The 3` terminus of genomic RNAs in highly conserved among other members of the family *Arenaviridae*, suggesting that these 19 terminal nucleotides (nt) may contain *cis* acting signals for the replication and transcription [10]. The intergenic regions (IGRs) in both L and S segments have the potential to form stable stem-loop (hairpin) structures [7, 11, 12]. Arenavirus transcribe subgenomic messager ribonucleic acid (RNA) for each of the five viral proteins [10]. The viral 3` ends of the mRNAs, which are nonpolyadenylated and heterologous, have been mapped to the base of the hairpin on the distal sides of [13, 14], suggesting a possible transcriptional regulatory role of the IGR [10].

1.1.1 **Arenavirus protein coding strategy:**

The S RNA directs synthesis of the four major structural proteins: the nucleoprotein, NP (ca. 63 kDa); two mature virion glycoproteins, GP-1 (40 to 46 kDa) and GP-2 (35 kDa), that are derived by posttranslational cleavage of a precursor polypeptide, GP-C (75 kDa) and a stable signal peptide (SSP) that functions in membrane fusion, SSP [4, 15] (see figure 1.2). The Nucleoprotein is the most abundant viral polypeptide both in infected cells and in virions (about 1530 NP molecules per virion particle) [16]. The NP and viral polymerase are complexed with the genomic viral RNA to form ribonucleoprotein (RNP) complexes, which are active in



virus transcription and replication [16].

The NP, the most abundant viral protein in virally infected cells, is associated with the viral RNA (vRNA) to form the nucleocapsid (NC) which is the template for the viral RNA polymerase [17]. The phosphorylated forms of the NP are usually detected at late stages of acute infection, and their abundance increases in persistently infected cells; however, the functional implications of these changes in the stage of NP phosphorylation have not been established [18].

Figure 1.2. Schematic illustration of the arenavirus replication process adopted from (http://education.expasy.org/images/Arenaviridae_genomes.jpg) accessed on 10-06-2011

The virion contains four structural proteins Glycoprotein GP-C (cleaved into Stable Single Peptide (SSP), GP-1, GP-2), Matrix protein Z and Nucleoprotein (NP): (i) the large cleaved transmembrane glycoprotein (GP), which is similar in organization to type I membrane fusion proteins [19]; (ii) a budding factor Z, which contains a metal-binding RING finger domain and regulates viral transcription and translation; (iii) the RNA binding nucleoprotein (NP), which is required for viral RNA polymerase activity; and (iv) a small, predominantly hydrophobic structural protein, organized similarly to the alphavirus 6K protein, that serves as a cleaved signal sequence for GP and is incorporated in the virion [20-22]. In addition, the viral replicase protein is incorporated at a low copy number.

1.2 Glycoprotein (GP):

The product of the GPC gene encoded by the S segment is posttranslationally cleaved into three mature proteins: SSP, GP-1 and GP-2. SSP, GP-1 and GP-2, as well as NP, L, and Z, are structural proteins present in virions. GP-1 and GP-2 make up the spikes on the virion envelope and mediate virus interaction with host cell surface receptor [23, 24]. Evidence indicated that GP-1 mediates virus interaction with host cell surface receptor, which has been recently identified as α-dystroglycan [24, 25]. Tetramers of GP-1 and GP-2 make up the spikes on the virion envelope.

The viral GPC (70-80 kDa) is posttranslationally proteolytically processed to generate the two mature virion glycoproteins, GP-1 (40-46 kDa) and GP2 (35 kDa) [26] [27], and a 58 amino acid signal peptide (SSP) [20, 22, 28] [20, 21]. Evidence indicate that in addition to its predicted role in targeting the nascent polypeptide to the endoplasmic reticulum, the SP of the arenavirus GPC likely serves additional roles in the biosynthesis, trafficking, and function of the viral envelope glycoproteins [28] [22] [20, 21]. GP-1 is located at the top of the spike, away from the membrane, and is held in place by ionic interactions with the N-terminus of the

transmembrane GP-2 that forms the stalk of the spike [29]. GP-1 is the virion attachment protein that mediates virus interaction with host cell surface receptors and subsequent virus cell entry via receptor-mediated endocytosis. Cleavage of GPC into GP-1 and GP-2 is mediated by the SKI-1/S1P cellular protease to yield alph-dystroglycan (α-DG) has been identified as the receptor for LCMV, LFV, and many other arenaviruses [30-32]. Evidence indicates that unprocessed arenavirus GPC can traffic to the cell surface, but the correct processing of the virus GPC into GP1 and GP2 by the SKI/S1P cellular protease is strictly required for the production of infectious particles that bud from the plasma membrane [26] [33]. Accordingly, growth of LCMV was severely impaired in cells deficient in SK1-1/S1P. Proteolytic processing of LCMV GPC also depends on the structural integrity of its cytoplasmic tail. Notably deletion of the three C-terminal amino acids KRR of LCMV GPC blocked its proteolytic cleavage [34]. Sequence comparison of the cytoplasmic tails of arenaviruses revealed the presence of a conserved three amino acid sequence WKR (Tryptophan, Lysine and Arginine) at the C terminus of LCMV-GP and LASV-GP. WKR sequence motifs are highly conserved in signal transducers and activators of transcription (STATs), a family of transcription factors activated in a variety of cytokine signaling pathways [35]. WKR motifs are crucial for dimerization of STATs, a requirement for DNA binding and activity in transcription. In analogy, WKR motifs present at the C-terminus of the GP of Old World arenavirus may play a role in GP oligomerization and/or in binding to a yet unknown cellular or viral protein that is crucial for the proper folding and processing of the GP [18, 35] (see figure 1.3).

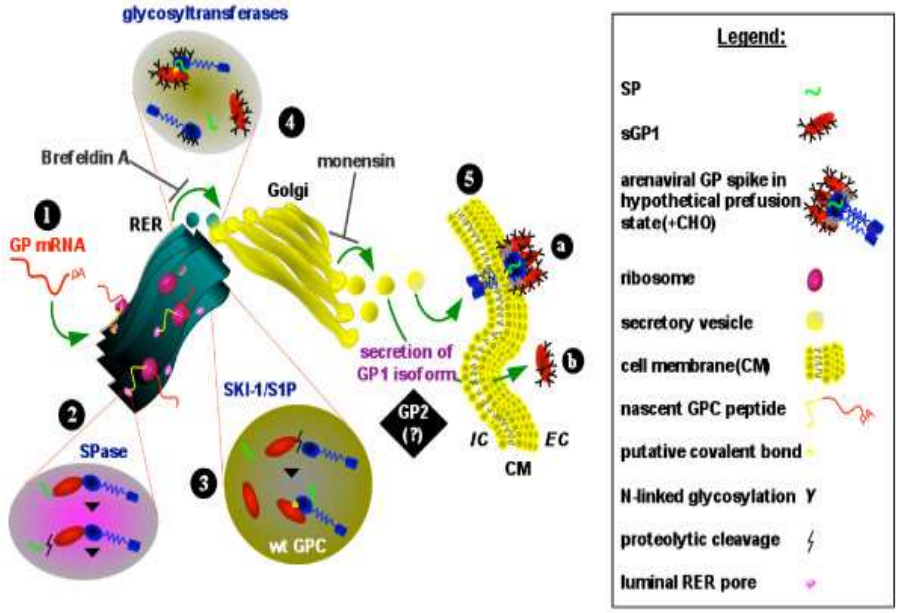


Fig 1.3 shows, in schematic presentation, the arenavirus glycoprotein expression pathway and secretary forms [36].

According to Bowden *et al* [37] although the MACV GP1 used for crystallization included residues 87 to 257, they do not see electron density for the last 20 C-terminal residues (the residues preceding the point of GP1/GP2 cleavage). MACV GP1 appears to be monomeric in the crystal. Structural database comparison [38] [39] suggests that MACV GP1 is composed of a fold that has not been previously observed. The N and C termini extend in the same direction, and the secondary structure consists of seven antiparallel β-strands; three α-helices, one of which is preceded by a 3_{10} helix; and two additional 3_{10} helices. When looking onto the edge of the β-sheet, the overall fold appears to be similar to the shape of a positive meniscus lens where the three large helices protect the convex side of the β-sheet whereas the concave side of then β-sheet is largely uncovered. The secondary structure is stabilized by four disulfide bonds which are present around the plane of the lens. Two of these disulfide bonds appear to be conserved across the New World HF arenaviruses, a third is also found in the GP1 of JUNV, while the fourth stabilizes a MACV-specific insertion. The presence of an additional conserved

disulfide bond between MACV GP1 and JUNV GP1 reflects the close relationship between these two viruses with respect to other New World arenaviruses [40] [41] [42]; thus, for the portion of the GP1 that Bowden *et al* [37] have analyzed, the sequence identity is 40%, and they have therefore expected the two structures to agree with approximately 1.2-Å root mean square deviation over matching Cα atoms [43]. The origins of the GPC fold are unknown; it bears no relation to the host ligand of TfR1, transferrin [44].

1.3 Z Matrix Protein:

The arenavirus small ring finger protein Z (ca 11 kDa), has no homolog among other known negative sense RNA viruses. Z is a structural component of the virion [45], in LCMV infected cells. Biochemical studies suggested that Z might be the arenavirus counterpart of the matrix (M) protein found in other NS RNA viruses [45]. The expression of Z during the progression from early to late phases of the LCMV life cycle appears to be highly regulated, and thereby Z might play different roles during the life cycle of LCMV. Its expression at low levels early during the life cycle could have a negative regulatory effect on RNA synthesis that might contribute to the restricted replication and noncytopathic properties of many arenaviruses. Increased levels of Z protein later during the LCMV life cycle could be required for virus assembly and budding.

The linear amino acid structure of Z could be seen as composed of three modules: the N-terminal part, the ring finger domain (RD), and the C-terminal part (figure 1.4). Despite some minor differences in protein size, the same over all structure is also seen among different arenavirus Z proteins. The Z ring domain exhibit the highest levels of conservation. The N-terminus contains a conserved canonical myristoylation motif [46], whereas the C terminus contains conserved proline motifs that are similar to those present within the L domains of several Gag and matrix viral proteins and that have been identified as key functional elements during budding of enveloped viruses from the plasma membrane [47] [48] [49] .

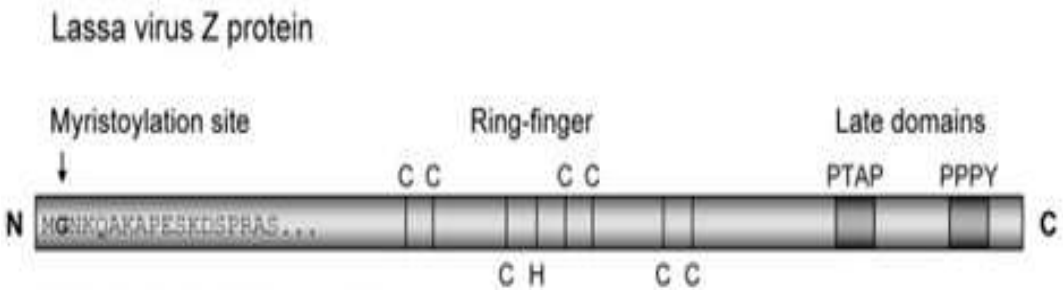


Figure 1.4. shows the schematic structure of the Z protein. Figure adopted from [50].

1.4 L Protein:

The L protein is thought to be the main viral component of the arenavirus polymerase [16]. The arenavirus L protein has the characteristic sequence motifs conserved among the RdRp (L proteins) of NS RNA virus. The proposed polymerase module of L is located within domain 3 which contains highly conserved amino acids within motifs designated A and C. Mutation function analysis showed that sequence SDD characteristics of motif C of segmented NS RNA virus, as well as the presence of the highly conserved D residue within motif A of L protein, are strictly required for the function of the LCMV polymerase [51].These studied revealed that many of mutant L proteins exhibited a strong dominant negative under assay conditions where the wild type and mutant L proteins did not compete for template RNA or other transacting proteins, viral or cellular, required for polymerase activity [51].These results were highly suggestive of L-L interaction being required for LCMV polymerase activity. Intragenic complementation has been documented for the L genes of several NS RNA viruses [52, 53].Consistent with this, direct L-L physical interaction has been demonstrated for the

paramyxoviruses Sendai [53] and Para influenza virus 3(PIV3) [54], this L oligomerization was required for polymerase activity. Likewise biochemical evidence supports that the LCMV L protein also has the property of forming oligomeric structure [51].

Detailed sequence analysis and secondary structure predictions have been documented for the LASV L polymerase [55]. These studies unequivocally identified only one functional domain corresponding to the putative RNA polymerase domain that exhibited a similar folding as those found in the corresponding domains of determined crystal structures of viral RdRp. In addition, several regions, outside the polymerase region, of strong alpha-helical content were identified, as well as putative coiled-coil domain at the N terminus. Secondary structure-assisted alignment of the RNA polymerase region indicated that arenaviruses are most closely related to the L protein of Nairoviruses [18].

1.5 Nucleoprotein (NP):

The Nucleoprotein (Mr 60-68kDA) is the most abundant viral polypeptide both in infected cells and in virions (about 1530 NP molecules per virion particle) [56]. The NP is the main structural element of the viral nucleocapsid and associates with the genome RNA to form beadlike structures [56].

The N terminal domain of NP adopts a completely novel fold not found in the Dali server. To identify the cap-binding residues in the deep cavity of the N domain, Qi *et al* attempted to soak and perform co-crystallization of LASV NP with m7GpppG, triphosphorylated, diphosphorylated or monophosphorylated ribonucleotides. They could observe the clear density for the triphosphate and partial density for uridine from the triphosphorylated ribonucleotide complex structures and they also visualized the structure of NP in complex with dTTP [57]. The triphosphate group of dTTP was bound in the middle of the cavity in an identical manner as that of UTP, in which it was anchored by salt bonds formed with the side chains of the conserved residues K309, R300, R323 and K253. In the deep end of the cavity, thymidine occupied a hydrophobic pocket that is composed of residues F176, W164, L172, M54, L120, L239 and I241. They proposed that this dTTP-binding pocket is the binding site for the cap structure m7GTP and that the residues located within the pocket may have to change conformation to accommodate the cap moiety [57]. Although the N domain of NP is not structurally similar to any of the cap-binding proteins, its hydrophobic thymidine-binding pocket shares common features for cap binding [58] [59]. To characterize the role of the cap-binding residues in viral RNA transcription, they [57] examined a panel of NP mutants with alanine substitution of residues located inside and at the entrance of the cavity and that are conserved among all known arenaviruses for their ability to mediate the cap-dependent viral RNA transcription using the LASV mini genome replicon assay [57]. All mutant proteins were expressed at similar levels as the wild type transfected with 15–30 ng of plasmid. Compared to the wild type, the K253A and E266A mutants completely lost the RNA transcription activity, and the Y319A, F176A, W164A, K309A and R323A mutants showed significantly decreased activity.

The C terminal domain of NP was reported by Qi *et al* to have exonuclease activity. Alanine substitution at five putative catalytic sites, D389A, E391A, D466A, D533A and H528A, in the mammalian cell expression vectors of either native or Myc-tagged NP gene, abolished exonuclease activity but did not affect viral protein expression in a minigenome system [60] [57] [57].

It was also examined by Qi *et al* whether the exoribonuclease activity is required for NP's function in the suppression of the interferon response [60, 61] [57]. As expected, wild type NP strongly inhibited Sendai-virus-induced interferon [beta] β activation by a promoter assay, whereas all the catalytic mutants D389A, E391A, D466A, D533A and H528A showed a complete loss of function at a low level of transfected expression vectors (10 ng) and showed various levels of deficiency at higher levels [57]. Their results confirm a previous study showing that the D389 residue of LASV NP, as well as its corresponding residue D382 in the prototypic

arenavirus lymphocytic choriomeningitis virus (LCMV), is required for IFN suppression but not for viral RNA transcription [62] [57]. In summary, these data provide strong genetic evidence for an important role of the NP exoribonuclease activity in suppressing interferon induction [57] (figure 1.5).

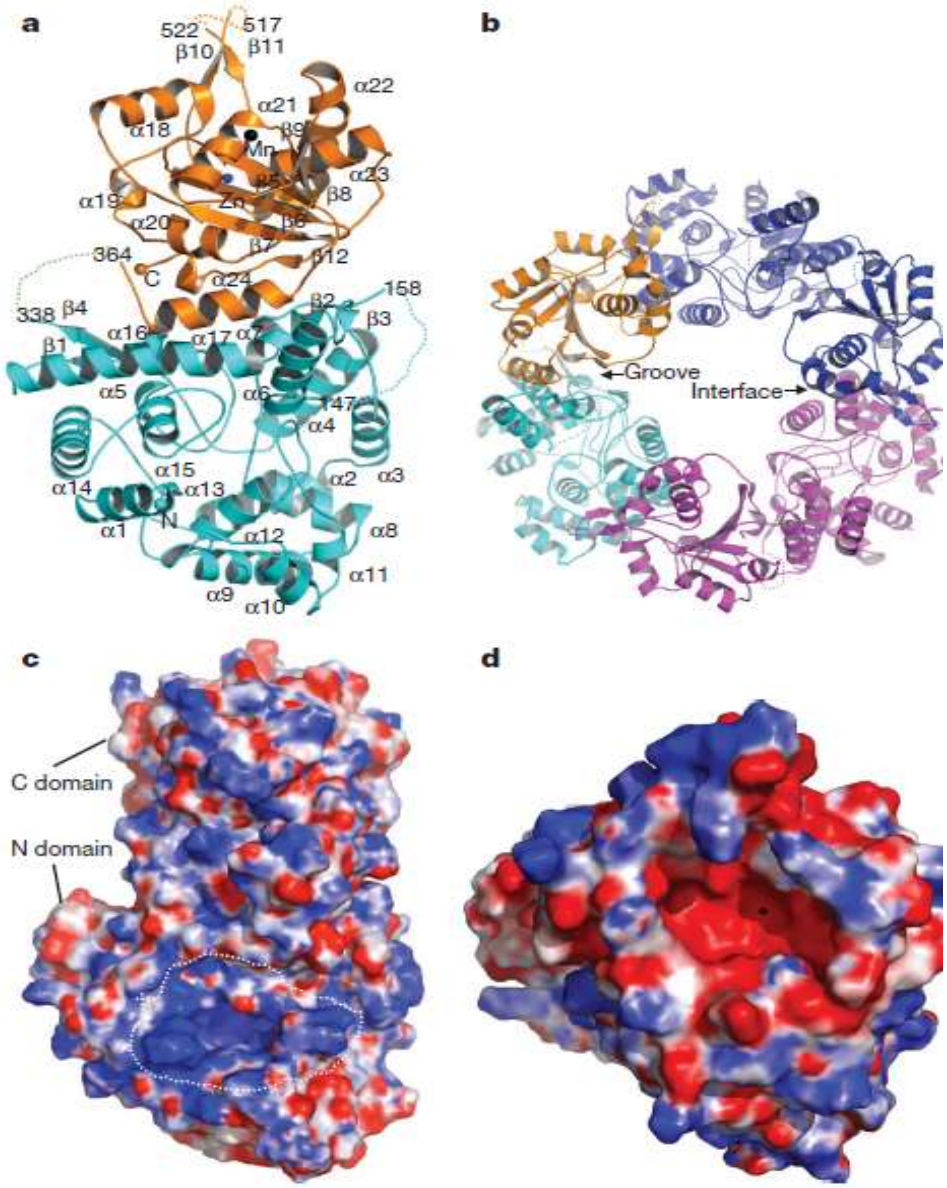


Figure 1.5. The crystal structure of LASV NP protein. a, Cartoon diagram of the LASV NP protomer. The N domain is in cyan with the cyan sphere indicating the N terminus; the C domain is in orange with the orange sphere indicating the C terminus.The black and blue spheres indicate the position of manganese and zinc ions respectively. The dotted lines represent the disordered loops. b, The ring-shaped structure of LASV NP trimer. The first protomer is coloured as in a, the second protomer is in blue and the third is in magenta. The groove and the interface are indicated by arrows. c, Electrostatic surface potential map of the NP protomer. The entrance of the cap-binding cavity is shown as a white dotted circle. The blue area represents positively charged residues and the red area represents negatively charged residues. d, Electrostatic surface potential map of the 39–59 exoribonuclease cavity. The black sphere represents Mn^{2+}. Figure adopted from Qi *et al* [57].

1.6 Replication:

Replication of arenavirus (figure 1.6) and influenza A virus appear to be similar so here a review of the better characterized influenza A virus replication is given. As in all negative-sense RNA viruses, the viral genomic RNA (vRNA) of influenza A virus is complexed with an RNA dependent RNA polymerase (comprising PB1, PB2, and PA subunits) and nucleoprotein (NP) into active viral ribonucleoprotein (vRNP), which serves as a template for both transcription and replication [63]. Whereas mRNA transcription requires capped RNA primers snatched from host pre-mRNA and premature poly (A) termination of transcripts, genome replication is primer independent and generates full-length cRNA. cRNA subsequently serves as the template for synthesis of progeny vRNA. Early studies [64] [65] [66] [67] suggested that replication requires *de novo* protein synthesis, and it was proposed that a "switch" regulates the transition from transcription to replication, but the molecular mechanism for such a switch has remained elusive despite more than two decades of research [66] [67].

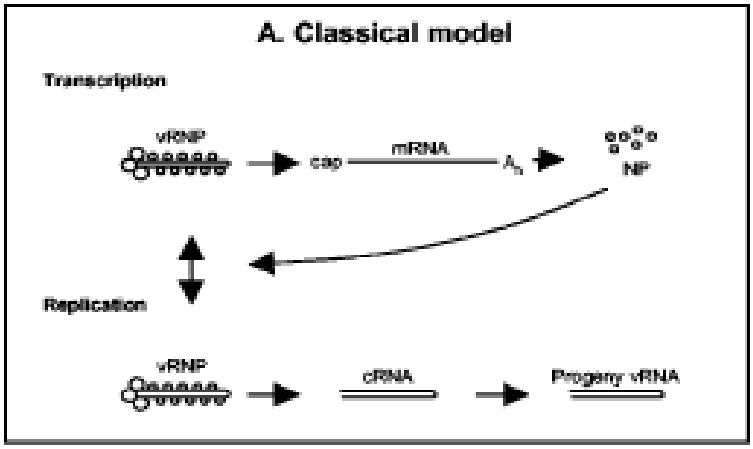


FIGURE. 1.6. Models for switching between influenza A viral transcription and replication. (A) Classical model. Early in infection, vRNA is transcribed to mRNA (primary transcription). Expression of NP switches primary transcription to replication (synthesis of cRNA and vRNA) and subsequent secondary transcription.

In arenavirus, the two RNA genomes are encapsidated by the NP, which is the most abundant protein in virions and infected cells, and act as templates for two fundamentally different processes, RNA replication and transcription. During RNA replication, the L protein first binds to the 39-end of RNA templates and reads them from end to end to direct the synthesis of encapsidated full-length anti-genomes [68]. During transcription, the RdRp stops RNA synthesis at a pause site located near the IGR [69]. The newly synthesized mRNA molecules have a nonpolyadenylated 3' end with a heterogeneous sequence mapped within the predicted hairpin in the IGR [70]. Furthermore, non-template-directed sequences have been identified at the 5′ end of the subgenomic mRNA [71]. These sequences are variable in length [70] [71] [72] and terminate with a 5'-cap structure, which suggests the presence of a cap-snatching mechanism for arenaviruses. In this process, originally described for influenza viruses [73] [74] and bunyaviruses [75], the viral RdRp binds cellular mRNAs caps and 'steals' them using an endonuclease activity, located in the influenza PA subunit [76] [77], and presumably in L protein of bunyaviruses and arenaviruses. These short capped RNAs are then used as primers for mRNA synthesis.

The arenavirus L protein is an essential element in genome replication and transcription [78]. It is the largest viral protein composed of approximately 2200 amino-acid (aa) residues, and sequence analysis using homologous proteins led to the prediction of several conserved domains [79] [55]. A biological function can be inferred for the L3 domain containing conserved and typical RdRp signature sequence motifs [55] [80]. For Tacaribe virus, both domains L1 and L3 interact with the Z protein [81]. By analogy with influenza and bunyaviruses, the L protein may also carry activities and domains responsible for a cap-snatching mechanism

that would account for the sequence diversity found at the 5'-end of RNA transcripts [68]. The expression and purification of such a large viral polymerase is problematic and has not been documented.

The N-terminal domain of the LCMV L protein has been shown to bind nucleotides, with a preference for UTP, and RNA. Structural comparison with the N-terminal part of the influenza virus PA protein unambiguously characterizes the domain as an endonuclease. Sequence and secondary structure analysis of L proteins from various Bunyaviridae family members predict that their N-terminal end carries a similar endonuclease activity, which has been demonstrated for Toscana virus (TOSV; genus Phlebovirus, family Bunyaviridae) [68] (figure 1.7).

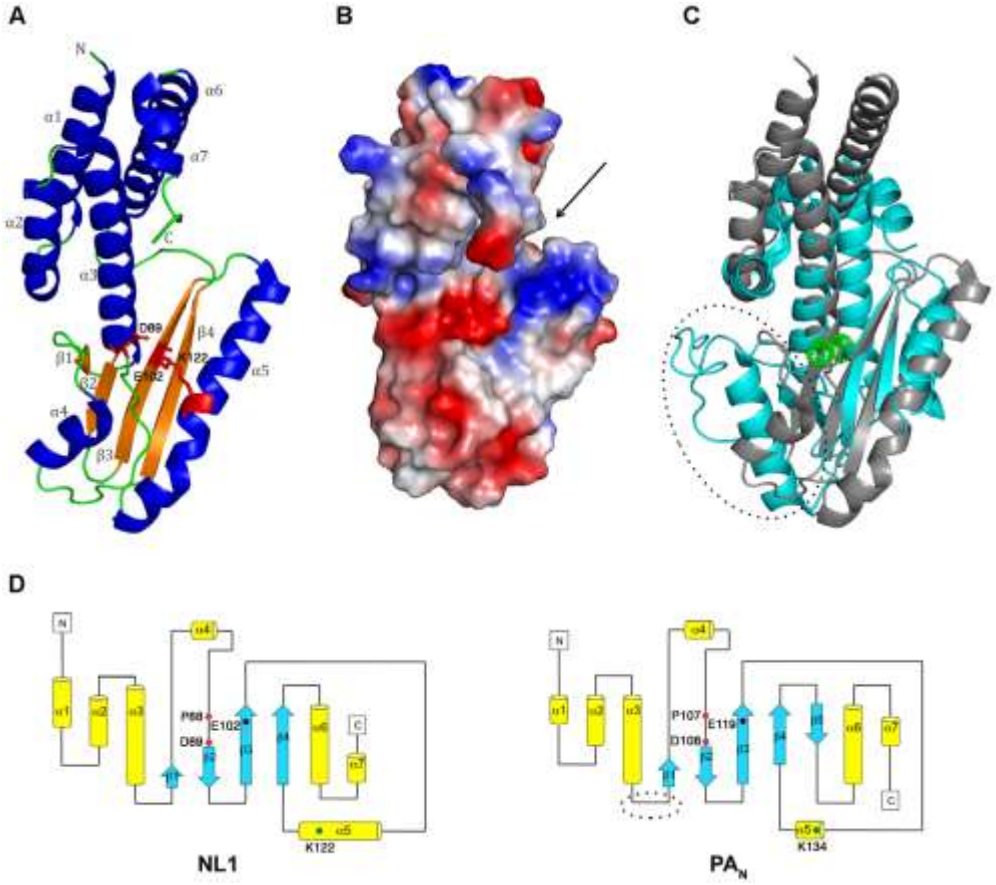


Figure 1.7 NL1 structure and comparisons with the influenza PA_N structure. A, Cartoon-representation of the NL1 structure. B, Electrostatic surface representation showing NL1 in the same orientation as in panel (A). The arrow indicates the putative RNA binding groove and the active site crevice. Negative charges are in red and positive charges in blue and neutral in white. C, Superimposition (view in the same orientation as in A) of the structures of NL1 (grey) and PA_N (PDB code:2W69, cyan) highlighting their shared structural core as well as variations in the form of an extra loop only present in the PAN structure (circled). The two Mn2+ ions in the PAN structure active site are depicted as green spheres. D, Topology diagrams of the NL1 (left) and PA_N (right) structures. Alpha-helices are represented as yellow tubes and beta-strands are blue arrows. Figure from Morin *et al* [68].

1.7 Budding:

The arenavirus Z protein is located on the inner side of the viral envelope, and despite being the smallest of the arenavirus proteins, with a size of only 90 to 99 amino acids, Z has been demonstrated to take part in a number

of processes and interactions [82]. It was first identified as a regulator of genome transcription and replication [83] [78] and has been shown to interact directly with the polymerase [84] [81]. Additional studies have shown numerous other interactions with cellular partners, including eukaryotic translation initiation factor 4E, promyelocytic leukemia protein, and the ribosomal protein [23] [85]. In addition to these regulatory functions, the Z protein of arenaviruses has, for several species, been shown to function as a matrix protein [86] [87] [47] [49] [88]. Characteristic for such proteins is the ability to mediate their own release in the form of virus-like particles (VLPs), which resemble virus particles in their morphology but lack the genomic segments [89]. Early studies suggested a role of Z in arenavirus transcriptional regulation [83]. However, it has been shown that Z is not required for virus RNA replication and transcription [90] [78]; rather, it exhibits a dose-dependent inhibitory effect on RNA synthesis mediated by the arenavirus polymerase [91] [78].

Both LCMV and LFV Z proteins exhibit self-budding activity [47] [49] and substituted efficiently for the late domain present in the Gag protein of Rous sarcoma virus [47]. These results indicate that Z is competent for both targeting to the plasma membrane and budding activities [47]. Consistent with its key role in arenavirus budding, Z accumulates near the inner surface of the plasma membrane [47]. Strong interaction of Z with cellular membranes has been observed [47] [49]. However, the mechanisms by which Z interacts and associates with the plasma membrane of the cell have not been determined.

Z is myristoylated at a conserved glycine found at position 2. A wide range of viruses express proteins that are myristoylated (reviewed in reference [92]). Myristoylation of N-terminal glycine residues of cellular and viral proteins changes the lipophilicity of these proteins and facilitates their interactions with membranes and hydrophobic protein domains. N-terminal sequence motifs that direct incorporation of fatty acids into proteins are necessary for targeting of these proteins to membranes and also have been shown to be required for the acquisition of the viral envelope by several RNA and DNA viruses [92]. The non-reversible covalent attachment of myristate, a 14-carbon saturated fatty acid, to the N-terminal glycine of myristoylated proteins is catalyzed by the myristoyl-coenzyme A:protein N-myristoyltransferase (NMT) [93] and occurs usually cotranslationally following removal of the initiator methionine by a methionine aminopeptidase [94]. Data from studies on both sequence of known myristoylated proteins and sequence requirements for NMT activity indicate that the N terminal acceptor amino acid is always a G residue [95]. Myristoylation alone does not meet the requirements for protein membrane targeting. Additional factors, such as the presence of basic amino acid residues that facilitate electrostatic interactions with membrane lipids, contribute to membrane association of myristoylated proteins [94]. Notably, comparison of 17 arenavirus Z protein sequences, including LCMV Z and those of HF arenaviruses, revealed the presence of a conserved glycine (G) residue on position 2 in the context of a consensus myristoylation signal [94]. In addition, clusters of basic amino acid residues are found within the N termini of arenavirus Z proteins [47].

It has been shown for many viruses including arenaviruses that the matrix protein is functionally dependent on the presence of late-domain motifs for self-budding activity (reviewed in references [96] [97] [48]. To date, four late-domain sequences have been reported, PPxY, PT/SAP, YxxL, and ѳPxV (where x is any amino acid and ѳ is a hydrophobic amino acid) [97] [98]. This also seems to be the case for several arenaviruses, although the number and sequence (s) of the late domains within Z vary. For the Old World arenavirus LCMV, a single C-terminal PPxY motif was shown to be responsible for budding [47], while for LASV both a PTAP motif and a PPxY motif were involved, with PPxY playing a dominant role [49]. In both cases, this process was dependent on the ESCRT (endosomal sorting complex required for transport) pathway [47] [99]. For the New World arenaviruses, a PT/SAP motif is present in all species, except for TCRV, in which only an ASAP motif is found at the corresponding position. It has been shown that JUNV Z is capable of mediating the production of chimeric VLPs containing both TCRV NP and JUNV GP and that budding of these particles is largely, although not entirely, dependent on the PTAP motif [86]. However, it remains to be shown whether these observations will hold true during the budding of nonchimeric VLPs made up of only TCRV or only JUNV proteins [82].

Interestingly, the TCRV ASAP motif was recently shown not to play a role in TCRV Z budding, although this process is still dependent on the ESCRT pathway [88]. In addition, New World arenaviruses also contain a YxxL motif, but to date, no function has been associated with this sequence; indeed, a recent report indicates that for TCRV, mutation of this motif has no effect on budding [88]. Cumulatively, these observations have led to the conclusion that TCRV seems to employ a budding strategy distinct from that of other arenaviruses via a process that seems to be independent of known late domains. Therefore, It has been compared the budding of JUNV and TCRV Z alone and in the presence of other viral proteins in order to identify their contributions to budding and provide new insight into the mechanism of budding in TCRV [82].

It has been postulated that NP may influence the formation of conformations of Z that are capable of budding more efficiently or may be required for efficient transport of Z to the budding site. Mutations in the ASAP and YxxL motifs of TCRV had been shown to be unimportant for budding of TCRV Z alone [88]. However, it has been shown that Z and NP together are necessary for efficient budding of TCRV. This is in contrast to the budding of closely related JUNV, which appears to be representative of the budding strategy of all other New World arenaviruses, as indicated by their common late-domain motif arrangement. It has been proposed that the ASAP motif, and the proline residue in particular, may be important for enhancement of TCRV Z budding by NP, possibly via interaction with NP [82] (figure 1.8).

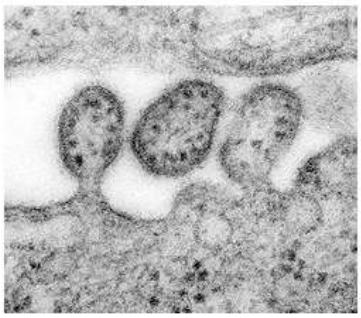


Figure 1.8 shows a photograph of a budding arenavirus particle. http://instruct1.cit.cornell.edu/research/whittakerlab/projects.html accessed on 21st June, 2011.

1.8 Structure of Virion:

All known animal viruses with helical nucleocapsids, such as arenavirus are enveloped [100, 101]. Arenavirus enveloped particles vary in diameter from 60 to more than 300 nm, with an average size of about 92 nm [100]. The virions consist of an envelope and a nucleocapsid [100, 101]. Electron cryomicroscopy (cryo-EM) has revealed that pleomorphic enveloped viruses have a roughly spherical appearance, studded with projections that correspond to oligomers of the attachment and fusion proteins. Examples include influenza virus [102] [103] [104]; several retroviruses, such as foamy virus [105], human immunodeficiency virus [106] [107] [108] [109] [110], murine leukemia virus [111], and Rous sarcoma virus [112] [113]; La Crosse virus [114] [115]; Sendai virus [116]; and transmissible gastroenteritis coronavirus [117].

Data Collection Methodology

Images of purified arenaviruses were generated prior to the inception of this project. Growth, purification, electron microscopy and images was carried out as described previously [120]. These methods are described briefly below.

2.1 Virus growth:

Arenaviruses were grown in Baby hamster kidney (BHK-21) cells (American Type Tissue Culture Collection); a virus stock, devoid of DI virus, was used in all experiments. BHK-21 cells were maintained as monolayer cultures in Dulbecco modified Eagle medium (DMEM), containing 20% fetal calf serum, 100 IU penicillin/ml, and 100 µg of streptomycin/ml (DMEM20).This all was done with the help of Dr John Burns [120]. JUNV-candid #1 was grown as described previously by Neuman *et al* [149].

2.2 Virus Purification:

Viruses were purified according to the method of Neuman et al [150]. Virions were harvested from infected cell culture medium by precipitation with 10% polyethylene glycol (PEG-8000). The PEG pellet was resuspended in 0.9% NaCl with 10 mM HEPES and that was applied to a 10 to 30% sucrose density gradient. Final purification was achieved by ultracentrifugation at 27,500 rpm for 2 h at 5°C. Virus particles were resuspended in HEPES-buffered saline which then were cryopreserved immediately (LCMV, PICV and TCRV), or fixed with 10% formalin overnight at 4°C prior to cryopreservation (JUNV) [120].

2.3 Fusion Activation (Removal of GP-1 from intact virions):

According to Neuman *et al* [120] purified 35S-labeled or unlabelled LCM, PICV, or TCRV virions resuspended in TNE (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA [pH 7.4]) were pelleted at 4°C in an Airfuge centrifuge (Beckman Instruments, Palo Alto, Calif.) for 13 min at 22 lb/in2 (~100,000 ×*g*). The pellets were resuspended in 1 M LiCl (pH 7.4) or 1 M NaCl (pH 5.0) and incubated for 1 h at 37°C. Control virus preparations were resuspended in TNE and incubated in parallel with the high-salt preparations. The virus samples were then loaded onto continuous 5 to 50% sucrose density gradients, and ultracentrifugation was performed in an SW 50.1 rotor for 18 h at 40,000 rpm. Gradients were fractionated by bottom puncture, and 300-µl fractions were collected. Gradient profiles were established by counting the radioactivity in aliquots of each fraction in scintillation fluid with a Beckman LS 1801 liquid scintillation counter. The protein composition of each fraction was determined by using sodium dodecyl sulfate–10% polyacrylamide reducing gels [151] [152] [120].

2.4 Small unilamellar vesicle preparation and microscopy:

Images of small unilamellar were measured as described in Neuman *et al* [149]. Small unilamellar vesicles (SUV) (DOPC/DOPG/DSPE-PEG2000-biotin/ C18-DiO, 87:10:2:1) DOPC (dioleoyl-sn-glycero-3-phosphocholine), DOPG 1,2–Dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)],1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl (polyethylene glycol)2000] (ammonium salt) (DSPE-PEG2000-biotin) 3,39-Dioctadecyloxacarbocyanine perchlorate (C18-DiO) [153].

The SUV sample was vortexed and pre extruded once through a polycarbonate membrane filter (pore size 400 nm) and subjected to several freeze/thaw cycles according to previous published protocols [154]. The sample was divided into two separate populations, which were extruded 11 times through a polycarbonate membrane filter with pore size 100 nm (SUV100) or 200 nm (SUV200) at pressures of 200 psi and 100 psi, respectively. Multilamellarity of the vesicle preparations was assayed using cryoTEM and yielded 8% of total preparation.

2.5 Cryo-Electron Microscopy:

According to Neuman that Low-dose Cryo-EM was performed at 120 kV and great care was taken to avoid the damage of specimen by reducing electron dose. Two methods were applied for the capturing of digital images of Cryo-EM. Virus particle negatives were digitized using a Zeiss SCAI microdensitometer and all other had been stored and captured directly via a CCD image sensor at a final resolution of 2.26 A/pixel using Leginon

[155]. The signal strength and signal-to-noise ratio differed subtly depending on recording methodology. Image contrast was inverted so that protein density should appear white. The density histogram for each image was normalized to a common median gray value prior to analysis [120].

Results

2.10 MEASUREMENT METHOD AND ANALYSIS OF ARENAVIRUS AND VESICLES STRUCTURE.

Each virion was measured using the Eman program. Circumference of round and elliptical particles was calculated according to the method of Ramanujan namely. This method of computing π is given by great mathematician Ramanujan. Ramanujan gave his own formula to calculate the value of π using circle. The method is given below : Let PQR be a circle with center O of which a diameter is PR. Bisect PO at H and let T is the point of trisection OR nearer R. Draw TQ perpendicular to PR and place the chord RS = TQ. Join PS, and draw OM and TN parallel to RS. Place a chord PK = PM, and draw the tangent Pl = MN. Join RL, RK and KL. Cut off RC = RH. Draw CD parallel to KL meeting RD at D. Then the square on RD will be equal to circle PQR approximately.

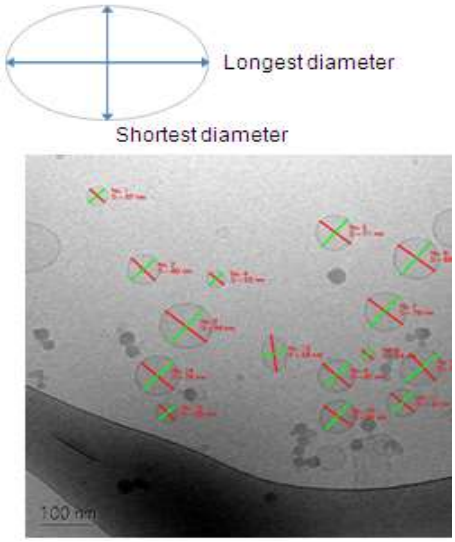


Figure 2.1. Size was taken as the circumference divided by π . The Ratio of the longest and shortest diameter tells about the shape of particles. Vesicle images courtesy of A. Kunding and D. Stamou, University of Copenhagen.

To make size data more readily comprehensible, the Ramanujan circumference was divided by π to convert circumference data to the effective particle diameter, if that particle was spherical. To calculate virus particle shape, the longest diameter was divided by the shortest, and the result was taken as a size-independent representation of virion shape.

Figure 2.1 illustrates the principle of virion measurement. By taking the measurement of the virion diameter in two places at right angles to each other the characteristic shape of the virion can be established.

2.11 Reproducibility of Measurement:

LCMV, PICV, TCRV, JUNV, and DOPC Vesicles were measured 2-4 times to quantify measurement variation. Standard deviation and standard error of both the data sets were calculated. The standard deviation Individual measurements were consistently ~8Å (about two image pixels). While the standard deviation for whole version measurements were ~1nm.

2.12 Measurement of Viral Glycoprotein (GP) spacing:

The estimated value of the average spacing distance between each GP was manually measured from the cryo-microscopic photographs by making use of the computer program EMAN [156].

From the analyzed results it was possible to extract the values for each GP density with respect to the specific diameter of a virion.

2.13 Glycoprotein (GP) Counting: GPs were counted using the EMAN program [156]. Each visible GP on visible outer edge of the virion was counted. This method was used for counting on all of virions of LCMV, TCRV, PICV and JUNV as well. By comparing the observed GP count to the theoretical number of GPs on a virion of a given size. It was possible to express GP coverage as a percent of the theoretical maximum.

2.14 Statistical tests used to analyze arenavirus data

2.15 Analysis of Variance (ANOVA):

Analysis of variance (ANOVA) is a collection of statistical models and their associated procedures in which the observed variance is partitioned into components due to different explanatory variables [159]. Simply ANOVA gives a statistical test of whether the means of several groups are equal, and therefore generalizes student's two-sample t-test to more than two groups [160]. ANOVAs are helpful because they possess a certain advantage over a two-sample t-test. Doing multiple two-sample t-tests would result in a largely increased chance of committing a 1 type error. For this reason, ANOVA are useful in comparing three or more means [159-161].

2.16 T Test: The *t*-statistic was introduced in 1908 by William Sealy Gosset, a chemist working for the Guinness brewery in Dublin Ireland ("Student" was his pen name) [162] [163] [164] Gosset had been hired due to Claude Guinness's policy of recruiting the best graduates from Oxford and Cambridge to apply biochemistry and statistics to Guinness' industrial processes [163].Gosset devised the *t*-test as a way to cheaply monitor the quality of stout. He published the test in Biometrika in 1908, but was forced to use a pen name by his employer, who regarded the fact that they were using statistics as a trade secret. In fact, Gosset's identity was known to fellow statisticians [165]. A two sample location test of the null hypothesis that the means of two normally distributed populations are equal. All such tests are usually called Student's *t*-tests, though strictly speaking that name should only be used if the variances of the two populations are also assumed to be equal; the form of the test used when this assumption is dropped is sometimes called Welch's *t*-test. These tests are often referred to as "unpaired" or "independent samples" *t*-tests, as they are typically applied when the statistical units underlying the two samples being compared are non-overlapping and were extensively used in this analysis [166].

2.17 Correlation coefficient (Pearson r):

It is widely used in the science as a measure of the strength of linear dependence between two variables [167]. It was developed by Karl Pearson from a similar but slightly different idea introduced Frances Galton in the 1880s [167]. In statistics the Pearson product- movement correlation coefficient is a measure of the correlation (linear dependence) between two variables X and Y, giving a value between +1 and -1 inclusive. The correlation coefficient is sometimes called "Pearson's r" [167, 168].

Conclusion: Simply ANOVA gives a statistical test of whether the means of several groups are equal, and therefore generalizes student's two-sample t-test to more than two groups [160]. ANOVAs are helpful because they possess a certain advantage over a two-sample t-test. Doing multiple two-sample t-tests would result in a largely increased chance of committing a 1 type error. For this reason, ANOVA are useful in comparing three or

more means [159-161]. The *t*-statistic was introduced in 1908 by William Sealy Gosset, a chemist working for the Guinness brewery in Dublin Ireland ("Student" was his pen name) [162] [163] [164] Gosset had been hired due to Claude Guinness's policy of recruiting the best graduates from Oxford and Cambridge to apply biochemistry and statistics to Guinness' industrial processes [163].Gosset devised the *t*-test as a way to cheaply monitor the quality of stout. He published the test in Biometrika in 1908, but was forced to use a pen name by his employer, who regarded the fact that they were using statistics as a trade secret. In fact, Gosset's identity was known to fellow statisticians [165]. A two sample location test of the null hypothesis that the means of two normally distributed populations are equal. All such tests are usually called Student's *t*-tests, though strictly speaking that name should only be used if the variances of the two populations are also assumed to be equal; the form of the test used when this assumption is dropped is sometimes called Welch's *t*-test.

Reference:

1. Salvato, M., Clegg JCS,Buchmeier MJ,Charrel RN, Gonzales JP,Lukashevish IS, Peters CJ, Rico-Hesse R, Romanoswski V, ed. *Family Arenaviridae*. ed. B.L. Van Regenmortel MHV. Vol. Eigth. 2005: Los Angless. 725-738.
2. Charrel, R.N. and X. de Lamballerie, *Zoonotic aspects of arenavirus infections.* Vet Microbiol, 2009.
3. Childs JE, P.C., *Ecology and Epidemiology of arenavirus and their hosts*, in *The Arenavirus*, M.S. Salvato, Editor. 1993, plenum Press: New York. p. 331-384.
4. Riviere, Y., et al., *The S RNA segment of lymphocytic choriomeningitis virus codes for the nucleoprotein and glycoproteins 1 and 2.* J Virol, 1985. **53**(3): p. 966-8.
5. Auperin, D.D., et al., *Sequencing studies of pichinde arenavirus S RNA indicate a novel coding strategy, an ambisense viral S RNA.* J Virol, 1984. **52**(3): p. 897-904.
6. Auperin, D.D., D.R. Sasso, and J.B. McCormick, *Nucleotide sequence of the glycoprotein gene and intergenic region of the Lassa virus S genome RNA.* Virology, 1986. **154**(1): p. 155-67.
7. Wilson, S.M. and J.C. Clegg, *Sequence analysis of the S RNA of the African arenavirus Mopeia: an unusual secondary structure feature in the intergenic region.* Virology, 1991. **180**(2): p. 543-52.
8. Romanowski, V. and D.H. Bishop, *Conserved sequences and coding of two strains of lymphocytic choriomeningitis virus (WE and ARM) and Pichinde arenavirus.* Virus Res, 1985. **2**(1): p. 35-51.
9. Salvato, M., E. Shimomaye, and M.B. Oldstone, *The primary structure of the lymphocytic choriomeningitis virus L gene encodes a putative RNA polymerase.* Virology, 1989. **169**(2): p. 377-84.
10. Conzelmann, K.K., *Genetic manipulation of non-segmented negative-strand RNA viruses.* J Gen Virol, 1996. **77 (Pt 3)**: p. 381-9.
11. Auperin, D.D. and J.B. McCormick, *Nucleotide sequence of the Lassa virus (Josiah strain) S genome RNA and amino acid sequence comparison of the N and GPC proteins to other arenaviruses.* Virology, 1989. **168**(2): p. 421-5.
12. Salvato, M.S. and E.M. Shimomaye, *The completed sequence of lymphocytic choriomeningitis virus reveals a unique RNA structure and a gene for a zinc finger protein.* Virology, 1989. **173**(1): p. 1-10.
13. Francis, S.J. and P.J. Southern, *Molecular analysis of viral RNAs in mice persistently infected with lymphocytic choriomeningitis virus.* J Virol, 1988. **62**(4): p. 1251-7.
14. Iapalucci, S., N. Lopez, and M.T. Franze-Fernandez, *The 3' end termini of the Tacaribe arenavirus subgenomic RNAs.* Virology, 1991. **182**(1): p. 269-78.
15. Southern, P.J., et al., *Molecular characterization of the genomic S RNA segment from lymphocytic choriomeningitis virus.* Virology, 1987. **157**(1): p. 145-55.
16. Fuller-Pace, F.V. and P.J. Southern, *Detection of virus-specific RNA-dependent RNA polymerase activity in extracts from cells infected with lymphocytic choriomeningitis virus: in vitro synthesis of full-length viral RNA species.* J Virol, 1989. **63**(5): p. 1938-44.
17. Fuller-Pace, F.V. and P.J. Southern, *Temporal analysis of transcription and replication during acute infection with lymphocytic choriomeningitis virus.* Virology, 1988. **162**(1): p. 260-3.
18. Buchmeier, M.J., *Arenaviruses: protein structure and function.* Curr Top Microbiol Immunol, 2002. **262**: p. 159-73.
19. Gallaher, W.R., C. DiSimone, and M.J. Buchmeier, *The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor.* BMC Microbiol, 2001. **1**: p. 1.

20. Eichler, R., et al., *Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor.* EMBO Rep, 2003. **4**(11): p. 1084-8.
21. Eichler, R., et al., *Signal peptide of Lassa virus glycoprotein GP-C exhibits an unusual length.* FEBS Lett, 2003. **538**(1-3): p. 203-6.
22. Froeschke, M., et al., *Long-lived signal peptide of lymphocytic choriomeningitis virus glycoprotein pGP-C.* J Biol Chem, 2003. **278**(43): p. 41914-20.
23. Borden, K.L., E.J. Campbell Dwyer, and M.S. Salvato, *An arenavirus RING (zinc-binding) protein binds the oncoprotein promyelocyte leukemia protein (PML) and relocates PML nuclear bodies to the cytoplasm.* J Virol, 1998. **72**(1): p. 758-66.
24. Borrow, P. and M.B. Oldstone, *Mechanism of lymphocytic choriomeningitis virus entry into cells.* Virology, 1994. **198**(1): p. 1-9.
25. Calain, P. and L. Roux, *The rule of six, a basic feature for efficient replication of Sendai virus defective interfering RNA.* J Virol, 1993. **67**(8): p. 4822-30.
26. Beyer, W.R., et al., *Endoproteolytic processing of the lymphocytic choriomeningitis virus glycoprotein by the subtilase SKI-1/S1P.* J Virol, 2003. **77**(5): p. 2866-72.
27. Pinschewer, D.D., et al., *Recombinant lymphocytic choriomeningitis virus expressing vesicular stomatitis virus glycoprotein.* Proc Natl Acad Sci U S A, 2003. **100**(13): p. 7895-900.
28. York, J., et al., *The signal peptide of the Junin arenavirus envelope glycoprotein is myristoylated and forms an essential subunit of the mature G1-G2 complex.* J Virol, 2004. **78**(19): p. 10783-92.
29. Buchmeier, M.J.B., M.D.,Peters,J.S., *Arenaviridae:The viruses and their replication.* 2001, Philadelphia: Williams and Wilkins. 1635-1668.
30. Cao, W., et al., *Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus.* Science, 1998. **282**(5396): p. 2079-81.
31. Kunz, S., P. Borrow, and M.B. Oldstone, *Receptor structure, binding, and cell entry of arenaviruses.* Curr Top Microbiol Immunol, 2002. **262**: p. 111-37.
32. Kunz, S., et al., *Molecular analysis of the interaction of LCMV with its cellular receptor [alpha]-dystroglycan.* J Cell Biol, 2001. **155**(2): p. 301-10.
33. Pinschewer, D.D., M. Perez, and J.C. de la Torre, *Role of the virus nucleoprotein in the regulation of lymphocytic choriomeningitis virus transcription and RNA replication.* J Virol, 2003. **77**(6): p. 3882-7.
34. Kunz, S., et al., *Mechanisms for lymphocytic choriomeningitis virus glycoprotein cleavage, transport, and incorporation into virions.* Virology, 2003. **314**(1): p. 168-78.
35. Ilaria, R.L., Jr., R.G. Hawley, and R.A. Van Etten, *Dominant negative mutants implicate STAT5 in myeloid cell proliferation and neutrophil differentiation.* Blood, 1999. **93**(12): p. 4154-66.
36. Branco, L.M. and R.F. Garry, *Characterization of the Lassa virus GP1 ectodomain shedding: implications for improved diagnostic platforms.* Virol J, 2009. **6**: p. 147.
37. Bowden, T.A., et al., *Unusual molecular architecture of the machupo virus attachment glycoprotein.* J Virol, 2009. **83**(16): p. 8259-65.
38. Holm, L., et al., *Searching protein structure databases with DaliLite v.3.* Bioinformatics, 2008. **24**(23): p. 2780-1.
39. Ye, Y. and A. Godzik, *Flexible structure alignment by chaining aligned fragment pairs allowing twists.* Bioinformatics, 2003. **19 Suppl 2**: p. ii246-55.
40. Bowen, M.D., C.J. Peters, and S.T. Nichol, *The phylogeny of New World (Tacaribe complex) arenaviruses.* Virology, 1996. **219**(1): p. 285-90.
41. Cajimat, M.N., et al., *Genetic diversity among Bolivian arenaviruses.* Virus Res, 2009. **140**(1-2): p. 24-31.
42. Charrel, R.N., et al., *Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination.* Biochem Biophys Res Commun, 2002. **296**(5): p. 1118-24.
43. Chothia, C. and A.M. Lesk, *The relation between the divergence of sequence and structure in proteins.* EMBO J, 1986. **5**(4): p. 823-6.
44. Bui, H.H., et al., *Protein sequence database for pathogenic arenaviruses.* Immunome Res, 2007. **3**: p. 1.
45. Salvato, M.S., et al., *Biochemical and immunological evidence that the 11 kDa zinc-binding protein of lymphocytic choriomeningitis virus is a structural component of the virus.* Virus Res, 1992. **22**(3): p. 185-98.
46. Perez, M., D.L. Greenwald, and J.C. de la Torre, *Myristoylation of the RING finger Z protein is essential for arenavirus budding.* J Virol, 2004. **78**(20): p. 11443-8.

47. Perez, M., R.C. Craven, and J.C. de la Torre, *The small RING finger protein Z drives arenavirus budding: implications for antiviral strategies.* Proc Natl Acad Sci U S A, 2003. **100**(22): p. 12978-83.
48. Freed, E.O., *Viral late domains.* J Virol, 2002. **76**(10): p. 4679-87.
49. Strecker, T., et al., *Lassa virus Z protein is a matrix protein and sufficient for the release of virus-like particles [corrected].* J Virol, 2003. **77**(19): p. 10700-5.
50. Strecker, T., et al., *The role of myristoylation in the membrane association of the Lassa virus matrix protein Z.* Virol J, 2006. **3**: p. 93.
51. Sanchez, A.B. and J.C. de la Torre, *Genetic and biochemical evidence for an oligomeric structure of the functional L polymerase of the prototypic arenavirus lymphocytic choriomeningitis virus.* J Virol, 2005. **79**(11): p. 7262-8.
52. Repik, P., A. Flamand, and D.H. Bishop, *Synthesis of RNA by mutants of vesicular stomatitis virus (Indiana serotype) and the ability of wild-type VSV New Jersey to complement the VSV Indiana ts G I-114 transcription defect.* J Virol, 1976. **20**(1): p. 157-69.
53. Smallwood S, C.B., Moyer SA,, *Intragenic complementation and oligomerization of the L sub unit of the Sendai Virus RNA polymerase* Virology, 2002. **304**(2): p. 235-245.
54. Smallwood, S. and S.A. Moyer, *The L polymerase protein of parainfluenza virus 3 forms an oligomer and can interact with the heterologous Sendai virus L, P and C proteins.* Virology, 2004. **318**(1): p. 439-50.
55. Vieth, S., et al., *Sequence analysis of L RNA of Lassa virus.* Virology, 2004. **318**(1): p. 153-68.
56. Buchmeier, M.J., J. C. de la Torre, and C. J. Peters, *Arenaviridae: The Viruses and Their Replication*. 2007, Philadelphia: Lippincott Williams & Wilkins.
57. Qi, X., et al., *Cap binding and immune evasion revealed by Lassa nucleoprotein structure.* Nature, 2010. **468**(7325): p. 779-83.
58. Guilligay, D., et al., *The structural basis for cap binding by influenza virus polymerase subunit PB2.* Nat Struct Mol Biol, 2008. **15**(5): p. 500-6.
59. Fechter, P. and G.G. Brownlee, *Recognition of mRNA cap structures by viral and cellular proteins.* J Gen Virol, 2005. **86**(Pt 5): p. 1239-49.
60. Martinez-Sobrido, L., et al., *Inhibition of the type I interferon response by the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus.* J Virol, 2006. **80**(18): p. 9192-9.
61. Martinez-Sobrido, L., et al., *Differential inhibition of type I interferon induction by arenavirus nucleoproteins.* J Virol, 2007. **81**(22): p. 12696-703.
62. Martinez-Sobrido, L., et al., *Identification of amino acid residues critical for the anti-interferon activity of the nucleoprotein of the prototypic arenavirus lymphocytic choriomeningitis virus.* J Virol, 2009. **83**(21): p. 11330-40.
63. Lamb, R.A., and R. M. Krug., *Orthomyxoviridae: the viruses and their replication,*, ed. F. In D. M. Knipe and P. M. Howley (ed.) and t.e. virology. 2001, Philadelphia, Pa.: Lippincott/The Williams & Wilkins Co. p. 1487–1530.
64. Barrett, T., A.J. Wolstenholme, and B.W. Mahy, *Transcription and replication of influenza virus RNA.* Virology, 1979. **98**(1): p. 211-25.
65. Hatada, E., et al., *Control of influenza virus gene expression: quantitative analysis of each viral RNA species in infected cells.* J Biochem, 1989. **105**(4): p. 537-46.
66. Hay, A.J., et al., *Transcription of the influenza virus genome.* Virology, 1977. **83**(2): p. 337-55.
67. Taylor, J.M., et al., *Use of specific radioactive probes to study transcription and replication of the influenza virus genome.* J Virol, 1977. **21**(2): p. 530-40.
68. Morin, B., et al., *The N-terminal domain of the arenavirus L protein is an RNA endonuclease essential in mRNA transcription.* PLoS Pathog, 2010. **6**(9).
69. Meyer, B.J., J.C. de la Torre, and P.J. Southern, *Arenaviruses: genomic RNAs, transcription, and replication.* Curr Top Microbiol Immunol, 2002. **262**: p. 139-57.
70. Meyer, B.J. and P.J. Southern, *Concurrent sequence analysis of 5' and 3' RNA termini by intramolecular circularization reveals 5' nontemplated bases and 3' terminal heterogeneity for lymphocytic choriomeningitis virus mRNAs.* J Virol, 1993. **67**(5): p. 2621-7.
71. Raju, R., et al., *Nontemplated bases at the 5' ends of Tacaribe virus mRNAs.* Virology, 1990. **174**(1): p. 53-9.
72. Polyak, S.J., S. Zheng, and D.G. Harnish, *5' termini of Pichinde arenavirus S RNAs and mRNAs contain nontemplated nucleotides.* J Virol, 1995. **69**(5): p. 3211-5.

73. Plotch, S.J., M. Bouloy, and R.M. Krug, *Transfer of 5'-terminal cap of globin mRNA to influenza viral complementary RNA during transcription in vitro.* Proc Natl Acad Sci U S A, 1979. **76**(4): p. 1618-22.
74. Plotch, S.J., et al., *A unique cap (m7GpppXm)-dependent influenza virion endonuclease cleaves capped RNAs to generate the primers that initiate viral RNA transcription.* Cell, 1981. **23**(3): p. 847-58.
75. Bishop, D.H., M.E. Gay, and Y. Matsuoko, *Nonviral heterogeneous sequences are present at the 5' ends of one species of snowshoe hare bunyavirus S complementary RNA.* Nucleic Acids Res, 1983. **11**(18): p. 6409-18.
76. Dias, A., et al., *The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit.* Nature, 2009. **458**(7240): p. 914-8.
77. Yuan, P., et al., *Crystal structure of an avian influenza polymerase PA (N) reveals an endonuclease active site.* Nature, 2009. **458**(7240): p. 909-13.
78. Lopez, N., R. Jacamo, and M.T. Franze-Fernandez, *Transcription and RNA replication of tacaribe virus genome and antigenome analogs require N and L proteins: Z protein is an inhibitor of these processes.* J Virol, 2001. **75**(24): p. 12241-51.
79. Muller, R., et al., *Rift Valley fever virus L segment: correction of the sequence and possible functional role of newly identified regions conserved in RNA-dependent polymerases.* J Gen Virol, 1994. **75 (Pt 6)**: p. 1345-52.
80. Lukashevich, I.S., et al., *The Lassa fever virus L gene: nucleotide sequence, comparison, and precipitation of a predicted 250 kDa protein with monospecific antiserum.* J Gen Virol, 1997. **78 (Pt 3)**: p. 547-51.
81. Wilda, M., et al., *Mapping of the tacaribe arenavirus Z-protein binding sites on the L protein identified both amino acids within the putative polymerase domain and a region at the N terminus of L that are critically involved in binding.* J Virol, 2008. **82**(22): p. 11454-60.
82. Groseth, A., et al., *Efficient budding of the tacaribe virus matrix protein z requires the nucleoprotein.* J Virol, 2010. **84**(7): p. 3603-11.
83. Garcin, D., S. Rochat, and D. Kolakofsky, *The Tacaribe arenavirus small zinc finger protein is required for both mRNA synthesis and genome replication.* J Virol, 1993. **67**(2): p. 807-12.
84. Jacamo, R., et al., *Tacaribe virus Z protein interacts with the L polymerase protein to inhibit viral RNA synthesis.* J Virol, 2003. **77**(19): p. 10383-93.
85. Campbell Dwyer, E.J., et al., *The lymphocytic choriomeningitis virus RING protein Z associates with eukaryotic initiation factor 4E and selectively represses translation in a RING-dependent manner.* J Virol, 2000. **74**(7): p. 3293-300.
86. Casabona, J.C., et al., *The RING domain and the L79 residue of Z protein are involved in both the rescue of nucleocapsids and the incorporation of glycoproteins into infectious chimeric arenavirus-like particles.* J Virol, 2009. **83**(14): p. 7029-39.
87. Eichler, R., et al., *Characterization of the Lassa virus matrix protein Z: electron microscopic study of virus-like particles and interaction with the nucleoprotein (NP).* Virus Res, 2004. **100**(2): p. 249-55.
88. Urata, S., J. Yasuda, and J.C. de la Torre, *The z protein of the new world arenavirus tacaribe virus has bona fide budding activity that does not depend on known late domain motifs.* J Virol, 2009. **83**(23): p. 12651-5.
89. Hoenen, T., L. Kolesnikova, and S. Becker., *Recent advances in filovirus and arenavirus-like particles.* Future Virol., 2007. **2**: p. 193–203.
90. Lee, K.J., et al., *NP and L proteins of lymphocytic choriomeningitis virus (LCMV) are sufficient for efficient transcription and replication of LCMV genomic RNA analogs.* J Virol, 2000. **74**(8): p. 3470-7.
91. Cornu, T.I. and J.C. de la Torre, *RING finger Z protein of lymphocytic choriomeningitis virus (LCMV) inhibits transcription and RNA replication of an LCMV S-segment minigenome.* J Virol, 2001. **75**(19): p. 9415-26.
92. Maurer-Stroh, S. and F. Eisenhaber, *Myristoylation of viral and bacterial proteins.* Trends Microbiol, 2004. **12**(4): p. 178-85.
93. Rajala, R.V., et al., *N-myristoyltransferase.* Mol Cell Biochem, 2000. **204**(1-2): p. 135-55.
94. Farazi, T.A., G. Waksman, and J.I. Gordon, *The biology and enzymology of protein N-myristoylation.* J Biol Chem, 2001. **276**(43): p. 39501-4.
95. Maurer-Stroh, S., B. Eisenhaber, and F. Eisenhaber, *N-terminal N-myristoylation of proteins: prediction of substrate proteins from amino acid sequence.* J Mol Biol, 2002. **317**(4): p. 541-57.
96. Bieniasz, P.D., *Late budding domains and host proteins in enveloped virus release.* Virology, 2006. **344**(1): p. 55-63.
97. Chen, B.J. and R.A. Lamb, *Mechanisms for enveloped virus budding: can some viruses do without an ESCRT?* Virology, 2008. **372**(2): p. 221-32.

98. Schmitt, A.P., et al., *Evidence for a new viral late-domain core sequence, FPIV, necessary for budding of a paramyxovirus.* J Virol, 2005. **79**(5): p. 2988-97.
99. Urata, S., et al., *Cellular factors required for Lassa virus budding.* J Virol, 2006. **80**(8): p. 4191-5.
100. Hassett, D.E., et al., *Immune responses following neonatal DNA vaccination are long-lived, abundant, and qualitatively similar to those induced by conventional immunization.* J Virol, 2000. **74**(6): p. 2620-7.
101. Milligan, R.A., A. Brisson, and P.N. Unwin, *Molecular structure determination of crystalline specimens in frozen aqueous solutions.* Ultramicroscopy, 1984. **13**(1-2): p. 1-9.
102. Booy, F.P., R.W. Ruigrok, and E.F. van Bruggen, *Electron microscopy of influenza virus. A comparison of negatively stained and ice-embedded particles.* J Mol Biol, 1985. **184**(4): p. 667-76.
103. Fujiyoshi, Y., et al., *Fine structure of influenza A virus observed by electron cryo-microscopy.* EMBO J, 1994. **13**(2): p. 318-26.
104. Shangguan, T., et al., *Morphological changes and fusogenic activity of influenza virus hemagglutinin.* Biophys J, 1998. **74**(1): p. 54-62.
105. Wilk, T., et al., *Specific interaction of a novel foamy virus Env leader protein with the N-terminal Gag domain.* J Virol, 2001. **75**(17): p. 7995-8007.
106. Briggs, J.G., T. Wilk, R. Welker, H.-G. Krau¨sslich, and S. D. Fuller, *Structural organization of authentic, mature HIV-1 virions and cores.* EMBO J., 2003. **22**: p. 22:1707–1715.
107. Fuller, S.D., et al., *Cryo-electron microscopy reveals ordered domains in the immature HIV-1 particle.* Curr Biol, 1997. **7**(10): p. 729-38.
108. Goto, T., et al., *Projection structures of human immunodeficiency virus type 1 (HIV-1) observed with high resolution electron cryo-microscopy.* J Electron Microsc (Tokyo), 1994. **43**(1): p. 16-9.
109. Nermut, M.V., et al., *Further evidence of icosahedral symmetry in human and simian immunodeficiency virus.* AIDS Res Hum Retroviruses, 1993. **9**(10): p. 929-38.
110. Wilk, T., et al., *Organization of immature human immunodeficiency virus type 1.* J Virol, 2001. **75**(2): p. 759-71.
111. Yeager, M., et al., *Supramolecular organization of immature and mature murine leukemia virus revealed by electron cryo-microscopy: implications for retroviral assembly mechanisms.* Proc Natl Acad Sci U S A, 1998. **95**(13): p. 7299-304.
112. Kingston, R.L., N.H. Olson, and V.M. Vogt, *The organization of mature Rous sarcoma virus as studied by cryoelectron microscopy.* J Struct Biol, 2001. **136**(1): p. 67-80.
113. Yu, F., et al., *Characterization of Rous sarcoma virus Gag particles assembled in vitro.* J Virol, 2001. **75**(6): p. 2753-64.
114. Talmon, Y., et al., *Electron microscopy of vitrified-hydrated La Crosse virus.* J Virol, 1987. **61**(7): p. 2319-21.
115. Wang, G.J., M. Hewlett, and W. Chiu, *Structural variation of La Crosse virions under different chemical and physical conditions.* Virology, 1991. **184**(1): p. 455-9.
116. Hosaka, Y. and T. Watabe, *Cryoelectron microscopy of vitrified Sendai virions.* J Virol Methods, 1988. **22**(2-3): p. 347-9.
117. Risco, C., et al., *The transmissible gastroenteritis coronavirus contains a spherical core shell consisting of M and N proteins.* J Virol, 1996. **70**(7): p. 4773-7.
118. Dubois-Dalcq, M., K. V. Holmes, B. Rentier, and D. W. Kingsbury., *Assembly of enveloped RNA viruses.* 1984, New York, N.Y.: Springer Verlag,.
119. Young, P.R., *Arenaviridae,* . In M. V. Nermut and A. C.
Steven (ed.), Animal virus structure. 1987, New York, N.Y.: Elsevier, New York, N.Y.
120. Neuman, B.W., et al., *Complementarity in the supramolecular design of arenaviruses and retroviruses revealed by electron cryomicroscopy and image analysis.* J Virol, 2005. **79**(6): p. 3822-30.
121. Sessa, G. and G. Weissmann, *Phospholipid spherules (liposomes) as a model for biological membranes.* J Lipid Res, 1968. **9**(3): p. 310-8.
122. Szoka, F., Jr. and D. Papahadjopoulos, *Comparative properties and methods of preparation of lipid vesicles (liposomes).* Annu Rev Biophys Bioeng, 1980. **9**: p. 467-508.
123. Walde, P. and S. Ichikawa, *Enzymes inside lipid vesicles: preparation, reactivity and applications.* Biomol Eng, 2001. **18**(4): p. 143-77.
124. Edidin, M., *The state of lipid rafts: from model membranes to cells.* Annu Rev Biophys Biomol Struct, 2003. **32**: p. 257-83.
125. Torchilin, V.P., *Recent advances with liposomes as pharmaceutical carriers.* Nat Rev Drug Discov, 2005. **4**(2): p. 145-60.

126. Edwards, K.A. and A.J. Baeumner, *Analysis of liposomes.* Talanta, 2006. **68**(5): p. 1432-41.
127. Edwards, K.A. and A.J. Baeumner, *Liposomes in analyses.* Talanta, 2006. **68**(5): p. 1421-31.
128. Gennis, R.B., *Biomembranes: Molecular Structure and Function.* 1989, New York.: Springer-Verlag.
129. Heimburg, T., *Thermal Biophysics of Membranes.* 2007, New York: John Wiley & Sons.
130. Cornelius, F., *Functional reconstitution of the sodium pump. Kinetics of exchange reactions performed by reconstituted Na/K-ATPase.* Biochim Biophys Acta, 1991. **1071**(1): p. 19-66.
131. Palmieri, F., et al., *Functional properties of purified and reconstituted mitochondrial metabolite carriers.* J Bioenerg Biomembr, 1993. **25**(5): p. 525-35.
132. New, R.R.C., *Liposomes: A Practical Approach*. 1990: Oxford University Press, Oxford.
133. Julicher, F. and R. Lipowsky, *Domain-induced budding of vesicles.* Phys Rev Lett, 1993. **70**(19): p. 2964-2967.
134. Dobereiner, H.G., et al., *Budding and fission of vesicles.* Biophys J, 1993. **65**(4): p. 1396-403.
135. Kas, J. and E. Sackmann, *Shape transitions and shape stability of giant phospholipid vesicles in pure water induced by area-to-volume changes.* Biophys J, 1991. **60**(4): p. 825-44.
136. Mui, B.L., et al., *Influence of transbilayer area asymmetry on the morphology of large unilamellar vesicles.* Biophys J, 1995. **69**(3): p. 930-41.
137. Svetina, S. and B. Zeks, *Membrane bending energy and shape determination of phospholipid vesicles and red blood cells.* Eur Biophys J, 1989. **17**(2): p. 101-11.
138. Oldstone, M.B., R. Ahmed, J. Byrne, M. J. Buchmeier, Y. Riviere, and P. and S. 41:70–74., *Virus and immune responses: lymphocytic choriomeningitis virus as a prototype model of viral pathogenesis.* Br. Med. Bull, 1985. **41**: p. 70-74.
139. Nathaanson, N., *Viral pathogenesis.* 1997.
140. Salazar-Bravo, J., L.A. Ruedas, and T.L. Yates, *Mammalian reservoirs of arenaviruses.* Curr Top Microbiol Immunol, 2002. **262**: p. 25-63.
141. Carter, M.F., N. Biswal, and W.E. Rawls, *Characterization of nucleic acid of pichinde virus.* J Virol, 1973. **11**(1): p. 61-8.
142. Trapido, H. and C. Sanmartin, *Pichinde virus, a new virus of the Tacaribe group from Colombia.* Am J Trop Med Hyg, 1971. **20**(4): p. 631-41.
143. Wattem, R., *Junin Virus*. 2004, Virginia Informatics Institute Verginia Tech.
144. Oakes, M.I., L. Kahan, and J.A. Lake, *DNA-hybridization electron microscopy tertiary structure of 16 S rRNA.* J Mol Biol, 1990. **211**(4): p. 907-18.
145. http://www.bbc.co.uk/dna/hub/A914302. *Cryo-Electron Microscopy*. [cited 2011 3rd June].
146. Frank, J., *Three-Dimensional Electron Microscopy of Macromolecular Assemblies.* 2006, New York: Oxford University Press.
147. van Heel M, G.B., Matadeen R, Orlova EV, Finn R, Pape T, Cohen D, Stark H, Schmidt R, Schatz M, Patwardhan A, *"Single-particle electron cryo-microscopy: towards atomic resolution."*. Q Rev Biophys, 2000(33): p. 307–69.
148. Robinson, A.L., Science, 1986(234): p. 821-822.
149. Neuman, B.W., et al., *Development of peptide-conjugated morpholino oligomers as pan-arenavirus inhibitors.* Antimicrob Agents Chemother, 2011. **55**(10): p. 4631-8.
150. Neuman, B.W., et al., *Purification and electron cryomicroscopy of coronavirus particles.* Methods Mol Biol, 2008. **454**: p. 129-36.
151. Burns, J.W. and M.J. Buchmeier, *Protein-protein interactions in lymphocytic choriomeningitis virus.* Virology, 1991. **183**(2): p. 620-9.
152. Laemmli, U.K., *Cleavage of structural proteins during the assembly of the head of bacteriophage T4.* Nature, 1970. **227**(5259): p. 680-5.
153. Kunding, A.H., et al., *A fluorescence-based technique to construct size distributions from single-object measurements: application to the extrusion of lipid vesicles.* Biophys J, 2008. **95**(3): p. 1176-88.
154. Patty, P.J. and B.J. Frisken, *The pressure-dependence of the size of extruded vesicles.* Biophys J, 2003. **85**(2): p. 996-1004.
155. Suloway, C., et al., *Automated molecular microscopy: the new Leginon system.* J Struct Biol, 2005. **151**(1): p. 41-60.

156. Ludtke, S.J., P.R. Baldwin, and W. Chiu, *EMAN: semiautomated software for high-resolution single-particle reconstructions.* J Struct Biol, 1999. **128**(1): p. 82-97.
157. Neuman, B.W., et al., *Supramolecular architecture of severe acute respiratory syndrome coronavirus revealed by electron cryomicroscopy.* J Virol, 2006. **80**(16): p. 7918-28.
158. R. Gonzales, R.W., *Digital Image Processing*. 1992: Addison-Wesley Publishing Company.
159. Anscombe, F.J., *"The Validity of Comparative Experiments"*. . Journal of the Royal Statistical Society. Series A (General) 1948. **3**: p. 181–211.
160. Bailey, R.A., *Design of Comparative Experiments.* 2008: Cambridge University Press.
161. Caliński, T.K., Sanpei *Block designs: A Randomization approach*. Vol. 1. 2000, New York: Springer-Verlag.
162. Mankiewicz, R., *The Story of Mathematics (Princeton University Press), p.158*, Princeton.
163. O'Connor, J.J.R., Edmund F.,, *"Student's t-test", MacTutor History of Mathematics archive*, St Andrews.: University of St Andrews.
164. Fisher Box, J., *"Guinness, Gosset, Fisher, and Small Samples". Statistical Science* 1987. **2**(1): p. 45-52.
165. TN, R., *"William Sealy Gosset and William A. Silverman: two "students" of science".* Pediatrics 116 (3): , 2005. **116**(3): p. 732.
166. Fadem, B., *High-Yield Behavioral Science (High-Yield Series).* 2008, Hagerstwon: Lippincott Williams & Wilkins.
167. Nicewander., J.L.R.a.W.A., *Thirteen ways to look at the correlation coefficient.* Vol. 1. 1988: The American Statistician.
168. Stigler, S.M., *"Francis Galton's Account of the Invention of Correlation"*. 4. 1989. 73–79.
169. P.R.Young, C.R.H.a., *Applied Virology: Structure and Variation among arenaviruses.*, ed. E.B.E. Kurstak. 1984, Orlando, Fla: Academic press
170. Murphy, F.A. and S.G. Whitfield, *Morphology and morphogenesis of arenaviruses.* Bull World Health Organ, 1975. **52**(4-6): p. 409-19.
171. Justines, G. and K.M. Johnson, *Immune tolerance in Calomys callosus infected with Machupo virus.* Nature, 1969. **222**(5198): p. 1090-1.
172. Webb, P.A., *Properties of Machupo virus.* Am J Trop Med Hyg, 1965. **14**(5): p. 799-802.
173. Earp, L.J., et al., *The many mechanisms of viral membrane fusion proteins.* Curr Top Microbiol Immunol, 2005. **285**: p. 25-66.
174. Smith, A.E. and A. Helenius, *How viruses enter animal cells.* Science, 2004. **304**(5668): p. 237-42.
175. Sieczkarski, S.B. and G.R. Whittaker, *Viral entry.* Curr Top Microbiol Immunol, 2005. **285**: p. 1-23.
176. Helenius, A., et al., *On the entry of Semliki forest virus into BHK-21 cells.* J Cell Biol, 1980. **84**(2): p. 404-20.
177. Marsh, M. and R. Bron, *SFV infection in CHO cells: cell-type specific restrictions to productive virus entry at the cell surface.* J Cell Sci, 1997. **110 (Pt 1)**: p. 95-103.
178. Burns, J.W., and M. J. Buchmeier. , *Glycoproteins of the arenaviruses,*. In M. S. Salvato (ed.), ed. The Arenaviridae. 1993, New York,N.Y.: Plenum Press,.
179. Kaufmann, B., et al., *Capturing a flavivirus pre-fusion intermediate.* PLoS Pathog, 2009. **5**(11): p. e1000672.
180. Di Simone, C., M.A. Zandonatti, and M.J. Buchmeier, *Acidic pH triggers LCMV membrane fusion activity and conformational change in the glycoprotein spike.* Virology, 1994. **198**(2): p. 455-65.
181. Kielian, M. and F.A. Rey, *Virus membrane-fusion proteins: more than one way to make a hairpin.* Nat Rev Microbiol, 2006. **4**(1): p. 67-76.
182. Skehel, J.J., et al., *Changes in the conformation of influenza virus hemagglutinin at the pH optimum of virus-mediated membrane fusion.* Proc Natl Acad Sci U S A, 1982. **79**(4): p. 968-72.
183. Heinz, F.X. and S.L. Allison, *Structures and mechanisms in flavivirus fusion.* Adv Virus Res, 2000. **55**: p. 231-69.
184. Feng, Y., et al., *HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor.* Science, 1996. **272**(5263): p. 872-7.
185. Eckert, D.M. and P.S. Kim, *Mechanisms of viral membrane fusion and its inhibition.* Annu Rev Biochem, 2001. **70**: p. 777-810.
186. Da Poian, A.T., F.A. Carneiro, and F. Stauffer, *Viral membrane fusion: is glycoprotein G of rhabdoviruses a representative of a new class of viral fusion proteins?* Braz J Med Biol Res, 2005. **38**(6): p. 813-23.
187. http://www.snaggledworks.com/em_for_dummies. *cryo-electron microscope for dummies*. 2004 2004 13-3-2012].
188. Cornu, T.I. and J.C. de la Torre, *Characterization of the arenavirus RING finger Z protein regions required for Z-mediated inhibition of viral RNA synthesis.* J Virol, 2002. **76**(13): p. 6678-88.

189. McIlhinney, R.A., *The fats of life: the importance and function of protein acylation.* Trends Biochem Sci, 1990. **15**(10): p. 387-91.
190. Delchambre, M., et al., *The GAG precursor of simian immunodeficiency virus assembles into virus-like particles.* EMBO J, 1989. **8**(9): p. 2653-60.
191. Gheysen, D., et al., *Assembly and release of HIV-1 precursor Pr55gag virus-like particles from recombinant baculovirus-infected insect cells.* Cell, 1989. **59**(1): p. 103-12.
192. Garrus, J.E., et al., *Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding.* Cell, 2001. **107**(1): p. 55-65.
193. Martin-Serrano, J., et al., *Divergent retroviral late-budding domains recruit vacuolar protein sorting factors by using alternative adaptor proteins.* Proc Natl Acad Sci U S A, 2003. **100**(21): p. 12414-9.
194. Strack, B., et al., *AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding.* Cell, 2003. **114**(6): p. 689-99.
195. von Schwedler, U.K., et al., *Functional surfaces of the human immunodeficiency virus type 1 capsid protein.* J Virol, 2003. **77**(9): p. 5439-50.
196. Basyuk, E., et al., *Retroviral genomic RNAs are transported to the plasma membrane by endosomal vesicles.* Dev Cell, 2003. **5**(1): p. 161-74.
197. Sfakianos, J.N. and E. Hunter, *M-PMV capsid transport is mediated by Env/Gag interactions at the pericentriolar recycling endosome.* Traffic, 2003. **4**(10): p. 671-80.
198. Sfakianos, J.N., R.A. LaCasse, and E. Hunter, *The M-PMV cytoplasmic targeting-retention signal directs nascent Gag polypeptides to a pericentriolar region of the cell.* Traffic, 2003. **4**(10): p. 660-70.
199. Blot, G., et al., *Targeting of the human immunodeficiency virus type 1 envelope to the trans-Golgi network through binding to TIP47 is required for env incorporation into virions and infectivity.* J Virol, 2003. **77**(12): p. 6931-45.
200. Lopez-Verges, S., et al., *Tail-interacting protein TIP47 is a connector between Gag and Env and is required for Env incorporation into HIV-1 virions.* Proc Natl Acad Sci U S A, 2006. **103**(40): p. 14947-52.
201. von Schwedler, U.K., et al., *The protein network of HIV budding.* Cell, 2003. **114**(6): p. 701-13.
202. Batonick, M., et al., *Interaction of HIV-1 Gag with the clathrin-associated adaptor AP-2.* Virology, 2005. **342**(2): p. 190-200.
203. Dong, X., et al., *AP-3 directs the intracellular trafficking of HIV-1 Gag and plays a key role in particle assembly.* Cell, 2005. **120**(5): p. 663-74.
204. Hurley, J.H. and S.D. Emr, *The ESCRT complexes: structure and mechanism of a membrane-trafficking network.* Annu Rev Biophys Biomol Struct, 2006. **35**: p. 277-98.
205. Slagsvold, T., et al., *Endosomal and non-endosomal functions of ESCRT proteins.* Trends Cell Biol, 2006. **16**(6): p. 317-26.
206. Hicke, L. and R. Dunn, *Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins.* Annu Rev Cell Dev Biol, 2003. **19**: p. 141-72.
207. Marmor, M.D. and Y. Yarden, *Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases.* Oncogene, 2004. **23**(11): p. 2057-70.
208. Katzmann, D.J., M. Babst, and S.D. Emr, *Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I.* Cell, 2001. **106**(2): p. 145-55.
209. Babst, M., et al., *Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting.* Dev Cell, 2002. **3**(2): p. 271-82.
210. Bosch, B.J., et al., *The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex.* J Virol, 2003. **77**(16): p. 8801-11.
211. Bonifaci, N., R. Sitia, and A. Rubartelli, *Nuclear translocation of an exogenous fusion protein containing HIV Tat requires unfolding.* AIDS, 1995. **9**(9): p. 995-1000.
212. Weissenhorn, W., et al., *The central structural feature of the membrane fusion protein subunit from the Ebola virus glycoprotein is a long triple-stranded coiled coil.* Proc Natl Acad Sci U S A, 1998. **95**(11): p. 6032-6.
213. Waning, D.L., et al., *Activation of a paramyxovirus fusion protein is modulated by inside-out signaling from the cytoplasmic tail.* Proc Natl Acad Sci U S A, 2004. **101**(25): p. 9217-22.
214. Hsu, M., A. Scheid, and P.W. Choppin, *Activation of the Sendai virus fusion protein (f) involves a conformational change with exposure of a new hydrophobic region.* J Biol Chem, 1981. **256**(7): p. 3557-63.
215. Chernomordik, L.V. and M.M. Kozlov, *Protein-lipid interplay in fusion and fission of biological membranes.* Annu Rev Biochem, 2003. **72**: p. 175-207.

216. Kahn, J.S., et al., *Recombinant Vesicular Stomatitis Virus Expressing Respiratory Syncytial Virus (RSV) Glycoproteins: RSV Fusion Protein Can Mediate Infection and Cell Fusion.* Virology, 1999. **254**(1): p. 81-91.
217. Tischler, N.D., et al., *Human and rodent humoral immune responses to Andes virus structural proteins.* Virology, 2005. **334**(2): p. 319-26.
218. Marriott, A.C., A.A. el-Ghorr, and P.A. Nuttall, *Dugbe Nairovirus M RNA: nucleotide sequence and coding strategy*. Virology, 1992. **190**(2): p. 606-15.
219. Klasse, P.J., R. Bron, and M. Marsh, *Mechanisms of enveloped virus entry into animal cells.* Advanced Drug Delivery Reviews, 1998. **34**(1): p. 65-91.