



Review

The Effects of Viral Structural Proteins on Acidic Phospholipids in Host Membranes

Ricardo de Souza Cardoso  and Akira Ono 

Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, MI 48109, USA; rdsc@umich.edu

* Correspondence: akiraono@umich.edu

Abstract: Enveloped viruses rely on host membranes for trafficking and assembly. A substantial body of literature published over the years supports the involvement of cellular membrane lipids in the enveloped virus assembly processes. In particular, the knowledge regarding the relationship between viral structural proteins and acidic phospholipids has been steadily increasing in recent years. In this review, we will briefly review the cellular functions of plasma membrane-associated acidic phospholipids and the mechanisms that regulate their local distribution within this membrane. We will then explore the interplay between viruses and the plasma membrane acidic phospholipids in the context of the assembly process for two enveloped viruses, the influenza A virus (IAV) and the human immunodeficiency virus type 1 (HIV-1). Among the proteins encoded by these viruses, three viral structural proteins, IAV hemagglutinin (HA), IAV matrix protein-1 (M1), and HIV-1 Gag protein, are known to interact with acidic phospholipids, phosphatidylserine and/or phosphatidylinositol (4,5)-bisphosphate. These interactions regulate the localization of the viral proteins to and/or within the plasma membrane and likely facilitate the clustering of the proteins. On the other hand, these viral proteins, via their ability to multimerize, can also alter the distribution of the lipids and may induce acidic-lipid-enriched membrane domains. We will discuss the potential significance of these interactions in the virus assembly process and the property of the progeny virions. Finally, we will outline key outstanding questions that need to be answered for a better understanding of the relationships between enveloped virus assembly and acidic phospholipids.



Citation: de Souza Cardoso, R.; Ono, A. The Effects of Viral Structural Proteins on Acidic Phospholipids in Host Membranes. *Viruses* **2024**, *16*, 1714. <https://doi.org/10.3390/v16111714>

Academic Editor: Ekaterina (Katya) Heldwein

Received: 13 September 2024

Revised: 23 October 2024

Accepted: 28 October 2024

Published: 31 October 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: influenza A virus (IAV); human immunodeficiency virus (HIV-1); acidic phospholipids; phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂); phosphatidylserine (PS); virus assembly; plasma membrane; lipid microdomains; IAV matrix protein-1 M1; IAV hemagglutinin; HIV-1 Gag

1. Introduction

One feature distinguishing enveloped from non-enveloped viruses is their absolute reliance on host cell membranes for particle assembly and release. During infection, some enveloped viruses utilize the membranes of intracellular compartments for assembly and budding, examples being dengue virus [1–3], oropouche virus [4,5], herpes simplex virus-1 [6], hepatitis B virus [7], and SARS-CoV-2 [8]. Others, such as respiratory syncytial virus [9–11], influenza virus [12–14], ebola virus [15–17], and human immunodeficiency virus-1 [18–20], use the plasma membrane as a platform for assembly. Notably, the interaction of structural proteins from these viruses with the plasma membrane could induce unique changes in the membrane microenvironment. While many studies of the relationships between viral replication and lipids focus on cholesterol and lipid rafts, an increasing number of studies have revealed that a diverse range of viruses from various families utilize or modulate acidic phospholipids to enhance virus replication, assembly, and budding.

This review will focus on the interactions between cellular acidic phospholipids and the structural proteins of two enveloped viruses that assemble at the plasma membrane, influenza A virus (IAV) and human immunodeficiency virus type 1 (HIV-1). Specifically,

we will discuss how IAV hemagglutinin (HA; for the purpose of this review, HA will be regarded as a structural protein), IAV matrix protein 1 (M1) (Figure 1a), and HIV-1 Gag (Figure 1b) interact with the plasma membrane and influence phospholipid dynamics. We will then discuss how the virus-induced reorganization of acidic phospholipid could modulate the virus assembly process or the properties of nascent progeny virions, ultimately favoring or preventing the infection cycle. Lastly, we will illuminate critical open questions regarding the association of HA, M1, and Gag with acidic phospholipids that can guide future investigations in the field of virus-membrane interactions.

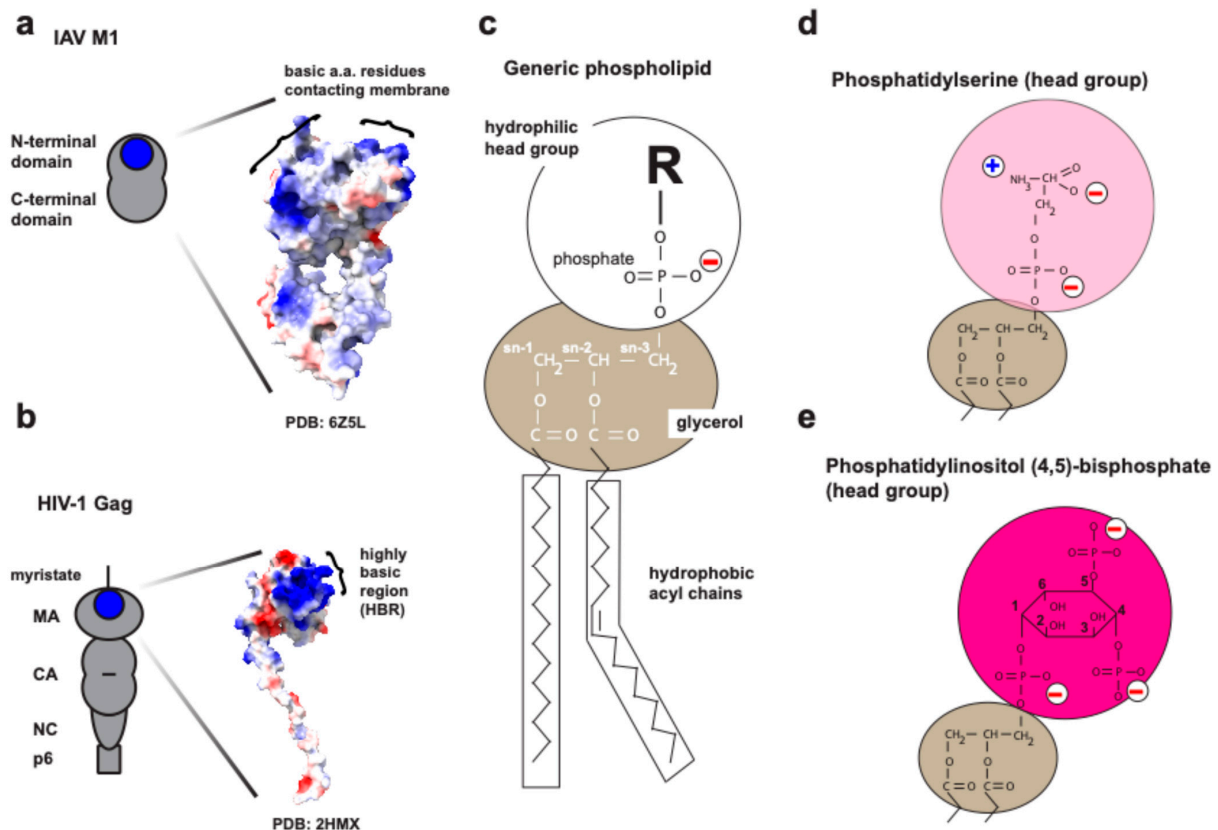


Figure 1. Structures of viral structural proteins and phospholipids. (a,b) Cartoon illustrations and structures of IAV M1 and HIV-1 Gag proteins. PDB accession numbers for the structures are shown at the bottom of each panel. Positively and negatively charged surfaces are indicated in blue and red, respectively, using UCSF ChimeraX software (v1.8). Basic residues involved in membrane binding are shown as blue circles in the cartoons and bracketed areas in the structures. (c) Schematic representation of a generic phospholipid structure. (d,e) Structures of the hydrophilic head group of phosphatidylserine (PS) and phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P₂]. Circles with a “−” or “+” symbol indicate the charges present in the PS and PI(4,5)P₂ head group.

2. Classification, Structure, and Cellular Distribution of Phospholipids

Phospholipids constitute a class of lipids that can be subdivided into glycerophospholipids and sphingomyelins (SM) [21,22]. Glycerophospholipids, which will be the main focus of the present review, have hydrophobic acyl chains at the sn-1 and -2 positions and a phosphate-containing head group at the sn-3 position of the glycerol backbone [23] (Figure 1c). Since their initial discovery in the early 1800s [24], various phospholipids have been identified, including the zwitterionic lipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), as well as the negatively charged lipids, phosphatidic acid (PA), phosphatidylserine (PS) (Figure 1d), and phosphatidylinositol (PI). The phosphatidylinositol lipids with a phosphorylated inositol headgroup (polyphosphoinositides) play diverse roles in cellular functions, which vary depending on the position(s) and the number of the

phosphate group on the inositol ring. Accordingly, they are distinguished as PI(3)P, PI(4)P, PI(5)P, PI(3,4)P2, PI(3,5)P2, PI(4,5)P2 (Figure 1e), and PI(3,4,5)P3 [25].

The intracellular distribution of the phospholipids varies between different organelles and within a single organellar membrane, thereby regulating the localization of lipid-binding proteins and hence, their specific functions [26–28]. The plasma membrane, a longitudinally non-homogeneous and transversally asymmetric organelle [27,29–31], is particularly enriched in negatively charged phospholipids [27] such as PA, PS, and PI(4,5)P2, which at physiological pH, have negative net charges of $-1/-2$, -1 , and -4 , respectively [25–27,32–34]. These acidic phospholipids interact with cations and/or proteins in a headgroup-specific and/or charge-dependent manner (Figure 1d,e), promoting membrane reorganization via protein/lipid clustering and membrane curvature [33,35–38]. Additionally, some of them, specifically polyphosphoinositides, are essential for numerous cellular signaling processes, such as the regulation of actin cytoskeleton dynamics and organization [25]. Importantly, acidic phospholipids play a pivotal role not only in cell biology but also in the assembly and egress of many viruses [39], wherein defects in lipid–viral protein interactions can culminate in the failure of efficient virus spread, as demonstrated for the Ebola [40–45], Nipah, and measles viruses [46], along with the viruses that we will discuss in this review, i.e., influenza A virus and HIV-1.

3. The Functions and Lateral Distributions of Plasma Membrane Acidic Phospholipids

An important feature of the plasma membrane is its high negative charge [47–49]. Experiments using polybasic probes demonstrated that the more positively charged the probe is, the more it binds to the plasma membrane relative to the intracellular membranous compartments [48]. The same study demonstrated that even after depletion of more highly charged PI(4,5)P2 or PI(3,4,5)P3, a subset of a polybasic probe binds to the plasma membrane, which contains PS, as detected by the lactadherin (LactC2) probe. The rest of the polybasic probes relocated to the intracellular compartments enriched in PS. These findings indicate that PS contributes to the negative charge of the plasma membrane, and that this charge contribution promotes the localization of positively charged proteins [48]. Because of its abundance at the plasma membrane and its negative charge (Figure 1d), PS has been suggested to play a role in the diverse cellular functions via its contribution to protein recruitment and clustering [26,48,50,51]. The cellular functions to which PS contributes include endo-, exo-, and phagocytosis and cell polarity maintenance [52–57].

Another acidic phospholipid that contributes to the negative charge of the plasma membrane is PI(4,5)P2 [58]. PI(4,5)P2 localization and maintenance at the plasma membrane is mediated by phosphoinositide kinases and phosphatases, inter-organellar trafficking via vesicles or membrane contact sites, and protein–lipid interactions [25,59–64].

The interaction between PI(4,5)P2 and proteins can be solely charge-dependent, as exemplified by myristoylated alanine-rich C-kinase substrate (MARKCS), which interacts with the acidic headgroup of the PI(4,5)P2 molecule (Figure 1e) via the positively charged effector domain [65–70]. Alternatively, the PI(4,5)P2 binding of a protein can be mediated by a combination of both structural- and charge-dependent interactions, as seen for some proteins containing the pleskstrin homology domain (PH) [69,71–75]. Other representative protein domains that associate with PI(4,5)P2 include Bin Amphiphysin Rvs (BAR) [69,76,77] and Epsin/AP180 N-Terminal Homology (ENTH/ANTH) domains [78,79], which are necessary for cellular functions, such as clathrin-mediated endocytosis [78,79].

Indeed, PI(4,5)P2 regulates clathrin-mediated endocytosis [60], from its initiation to its completion, by recruiting multiple proteins at multiple steps [73,80]. Subunits of the clathrin adapter protein complexes interact, via their basic residues, with PI(4,5)P2, which triggers their conformational changes and subsequent membrane deformation, leading to the coated pit formation [81]. Interestingly, this interaction can stimulate phosphatidylinositol phosphate kinases (PIPKs) to produce a PI(4,5)P2 pool dedicated to clathrin endocytosis [82]. Interactions of PI(4,5)P2 with the PH domain of dynamin, a GTPase, are essential to cause

the enrichment of dynamin in the budding membrane necks, which promotes pinching off and the release of the endocytic vesicle [80].

Local PI(4,5)P₂ enrichment can occur through additional mechanisms other than the PIPKs stimulation mentioned above. Studies have shown that the phagocytosis event is marked by the accumulation of PI(4,5)P₂, which induces actin nucleation and rearrangement at the phagosomal cup, and by subsequent redistribution of PI(4,5)P₂ to other plasma membrane areas [53,83–86]. A study using fluorescence correlation spectroscopy and fluorescence recovery after photobleaching approaches revealed that the retention of PI(4,5)P₂ is more likely due to molecular fences that restrict the escape of locally synthesized PI(4,5)P₂ than to reduced diffusion inside the phagosomal cup [85].

Fusion, the final step in the exocytosis mechanism, also involves PI(4,5)P₂ as a major player [87]. The fusion process is mediated by the SNARE complex, some of which consist of Syntaxin-1 and SNAP-25 [87]. Syntaxin-1, a transmembrane protein that localizes at the plasma membrane of the cells, has a juxtamembrane polybasic sequence (JMPBS) essential for the interaction with PI(4,5)P₂ [88–90]. Interestingly, this interaction is able to induce the co-clustering of syntaxin-1 and PI(4,5)P₂, which is distinguishable from the rest of the membrane [91].

In the cellular processes described above, PI(4,5)P₂ serves as a molecule that recruits effector proteins to the specific site of the plasma membrane and often does so through local enrichment. Therefore, whether the proteins are recruited to pre-existing PI(4,5)P₂ clusters or whether PI(4,5)P₂ clusters are a result of their recruitment by proteins has been intensely studied [33,37,92,93]. Notably, two-thirds of plasma membrane PI(4,5)P₂ is likely reversibly bound to proteins in the cells. This is supported by the significantly lower diffusion coefficient for fluorescent PI(4,5)P₂ in the inner leaflet of the plasma membrane versus that in the protein-free liposome membrane [94]. Corroborating this possibility, PI(4,5)P₂ can be found as clusters due to its association with basic residues of proteins [68,91,95,96].

As observed for host cellular proteins, viral structural proteins can alter the distribution of acidic phospholipids during virus assembly, whereas the distribution of acidic phospholipids can determine the site of virus assembly and the nature of nascent virus particles. Below, we will address the interplay between the acidic phospholipids and virus assembly, with a focus on the assembly of influenza and HIV virus particles.

4. Influenza A Virus (IAV) Assembly and Acidic Phospholipids

4.1. Influenza A Virus Assembly

The assembly of the influenza A virus (IAV) takes place at the plasma membrane of the infected cells. This process is orchestrated by its structural proteins, hemagglutinin (HA), neuraminidase (NA), matrix protein 1 (M1) (Figure 1a), matrix protein 2 (M2), and viral ribonucleoprotein complexes (vRNPs) [12,97,98]. The type I transmembrane protein hemagglutinin (HA), which is initially synthesized as the precursor HA0 [99], and the neuraminidase (NA), a type II transmembrane protein, reach the IAV assembly sites at the apical plasma membrane through the secretory pathway [12]. At the assembly sites, the acylation of the HA cytoplasmic tail promotes both HA-M1 interaction and membrane curvature at the assembly/budding sites [100]. NA plays a crucial role as a sialidase, cleaving the virus receptor sialic acid to facilitate the release of nascent IAV particles [101].

The M1 protein facilitates the export of the viral ribonucleoprotein complexes (vRNPs) from the nucleus to the cytosol [98,102]. At the plasma membrane, M1 forms a lattice underneath the lipid bilayer, forming the viral envelope, where it mediates the association of the viral transmembrane proteins, HA, NA, and M2, with the vRNPs [103]. Notably, the trafficking of M1 to the assembly sites depends on the presence of M2; the absence of M2 results in M1 mislocalization [104].

The assembly of HA, NA, M1, M2, and vRNPs complexes into nascent virus particles is coupled to particle budding at the plasma membrane [97]. The precise molecular mechanisms underlying the influenza budding steps are yet to be fully elucidated. However, several non-mutually exclusive mechanisms may contribute to the formation of membrane

curvature, which include: (1) influenza protein accumulation, which bends the membrane, either via molecular crowding or through forming a curve-inducing molecular shape; (2) the accumulation of cone-shaped lipids in the inner leaflet of the assembly sites; and (3) the involvement of the cytoskeleton or other host proteins that alter the membrane structures [97]. Unlike HIV or many other enveloped viruses, which utilize the endosomal sorting complex required for transport (ESCRT) machinery for the release of virus particles, the release of influenza virus particles from the plasma membrane occurs in an ESCRT-independent manner [105]. During the late events in assembly, M2 protein localizes to the neck of the budding particle, facilitating the budding/pinching off process to release the virus.

The IAV infection modulates the cellular lipid metabolism [106–111]. In addition, it has long been known that during the IAV assembly, the plasma membrane gives rise to specialized membrane microdomains known as budozones [12,112–115]. The budozones are thought to consist of lipid raft-like microdomains enriched in cholesterol and sphingolipids where several IAV proteins associate with it [116,117]. Early evidence has shown that HA [118], NA [119,120], and M1 [121] partition into detergent-resistant membranes (DRM), which is consistent with, but not the indication of, lipid raft association. In contrast, M2 is thought to localize at the boundary between the raft and non-raft domains [12,122]. Consistent with this notion, immuno-electron microscopy of the plasma membrane showed that M2 is present outside of the HA-rich domains [123]. The IAV infection increases the lipid packing of the infected cell plasma membrane and causes lower lateral diffusion of membrane-associated proteins [124]. Therefore, the IAV infection causes changes in the membrane dynamics at the whole cell and the membrane microdomain levels, both of which could modulate assembly and budding [108,114,117,124–127].

Accumulating evidence has shown that in addition to the lipid raft-like microdomains, acidic phospholipids are host determinants regulating IAV assembly and budding [128], but they are also modulated by infection [124]. In the next section, we will focus on how IAV HA and M1 proteins interact with and modulate the acidic phospholipids during IAV assembly.

4.2. The Interplay Between Acidic Phospholipids and Influenza A Virus Structural Proteins

4.2.1. Hemagglutinin (HA) and the Acidic Phospholipids

Fluorescence photoactivation localization microscopy (FPALM) of both living and fixed cells has revealed the presence of dynamic HA clusters at the plasma membrane, spanning in size from a few nanometers to a micrometer scale [129]. As alluded to earlier, it has been proposed that the lateral distribution of HA at the plasma membrane is determined by HA partitioning into cholesterol-enriched raft-like microdomains [112,123]. In support of this possibility, immunoelectron microscopy and FRET experiments showed that cholesterol depletion disrupts HA clustering and HA–raft marker association [123,130,131]. However, despite the cholesterol enrichment in IAV virions [117,125], a high-resolution secondary ion mass spectrometry (SIMS) study did not find a similar distribution of cholesterol inside and outside of the HA clusters at the plasma membrane [132]. Therefore, the exact role of cholesterol and the cholesterol-enriched microdomains in the formation of HA clusters remains to be elucidated [133].

Besides cholesterol, acidic phospholipids, such as PS and PI(4,5)P₂, can be found in the membrane fractions that were thought to represent lipid rafts [134–136], and hence, it is conceivable that these lipids may play a role in HA partitioning/clustering at the plasma membrane. Consistent with the possibility that HA associates with PI(4,5)P₂ at the plasma membrane, super-resolution experiments have shown that the motility of PI(4,5)P₂ decreases in cells expressing the influenza protein, and that elevated HA expression correlates with increased PI(4,5)P₂ clustering [137,138].

It was further shown that cetylpyridinium chloride (CPC), containing a monocationic head group, decreases HA clustering at the plasma membrane and reduces IAV loads and mortality in zebrafish [128]. CPC appears to act on PI(4,5)P₂, as FPALM experiments

demonstrate a decreased association between HA and a PI(4,5)P2 probe (GFP-tagged PH domain) in the presence of CPC. Additionally, the PM displacement of MARCKS, a PI(4,5)P2-binding protein, was observed in the presence of CPC [128]. These data collectively indicate that CPC abrogates the interaction between PI(4,5)P2 and HA. Altogether, the accumulating evidence suggests that PI(4,5)P2 may play a pivotal role in HA clustering, setting the stage for virus assembly and budding.

At present, it remains to be determined whether HA interacts directly or indirectly with acidic phospholipids. In vitro evidence favoring a possible direct association includes circular dichroism studies that demonstrated an increase in the helicity of an HA transmembrane peptide in membrane bilayers containing negatively charged lipids compared to the results for bilayers composed solely of zwitterionic lipids like PC [139]. The juxtamembrane C-terminal region in the HA protein of some IAV strains contains lysine or arginine residues [139,140] (Figure 2a), and since HA protein forms trimers, this can increase the positive charge in its cytoplasmic tail, potentially facilitating interactions with acidic phospholipids such as PS and PI(4,5)P2 (Figure 2b). Consistent with this possibility, a recent all-atom molecular dynamics (AAMD) simulation showed interactions between arginines in the HA cytoplasmic tail and phosphate groups in the PI(4,5)P2 head group [141]. Alternatively or additionally, HA may interact with PI(4,5)P2 indirectly through other proteins or protein complexes such as the actin cytoskeleton, which is regulated by PI(4,5)P2 and implicated in HA plasma membrane clustering [142].

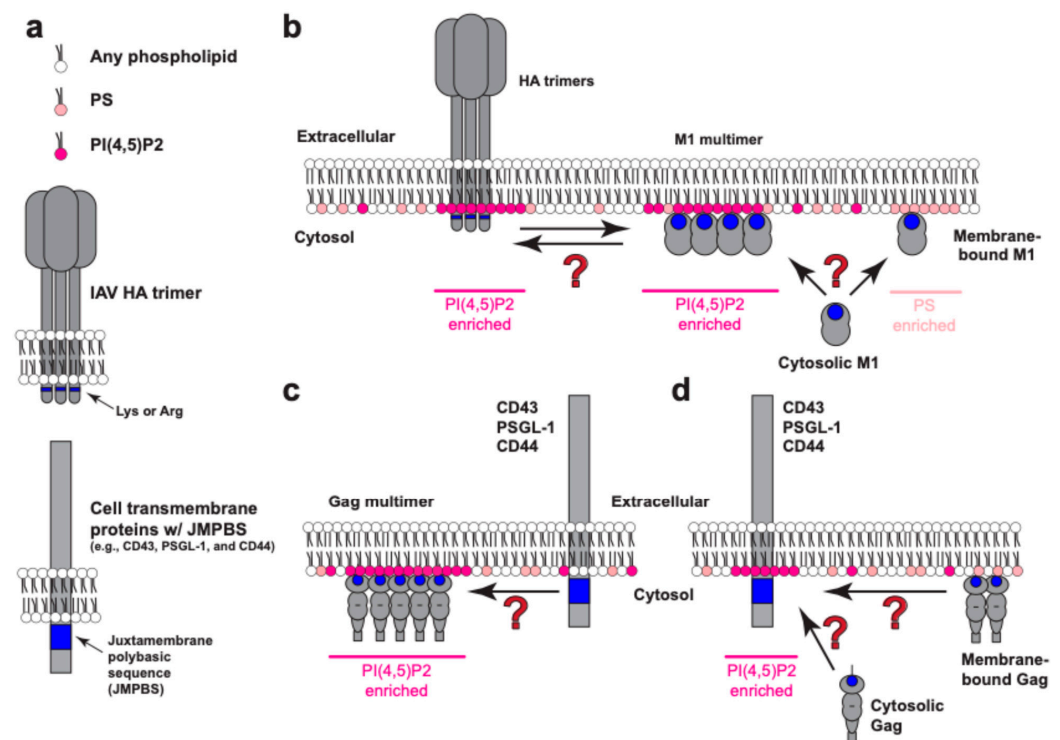


Figure 2. Potential mechanisms for viral and host transmembrane protein sorting at assembly sites mediated by acidic phospholipids. (a) Cartoon representation of phospholipids, IAV HA trimers, and host transmembrane proteins illustrating their association with the membrane. Basic residues of HA and the juxtamembrane polybasic sequences (JMPBS) are shown in blue. (b) HA trimers or M1 multimers associate with PI(4,5)P2-enriched membrane domains leading to the recruitment of each other. M1 also interacts with PS-enriched membrane domains, and this association may precede localization to the PI(4,5)P2-enriched domain. (c) HIV-1 Gag is localized in a PI(4,5)P2-enriched membrane domain, to which cellular transmembrane proteins with a JMPBS are recruited. (d) JMPBS of cellular proteins are present in a PI(4,5)P2-enriched membrane domain, which recruits cytosolic or plasma membrane-associated Gag.

4.2.2. Matrix Protein-1 (M1) and the Acidic Phospholipids

The N-terminal region of the M1 protein is a globular structure consisting of nine alpha-helices, and some of them contain basic residues that favor its electrostatic association with phospholipids [143–150] (Figure 1a). In addition, due to its multimerization property [12,151–153], M1 protein can increase its positively charged surface per oligomer, thereby increasing its affinity for plasma membrane acidic phospholipids. Several studies, which rely mostly on bulk biochemical approaches, have shown that M1 associates with PS [133,147,153]. Studies using FRET and small-angle X-ray scattering (SAXS) have demonstrated that the M1 protein can increase or stabilize the clustering of PS in small unilamellar vesicles (SUVs) [154,155]. Consistent with the *in vitro* studies, a fluorescent protein fusion of M1 and the fluorescent analogue of PS were observed to colocalize at the plasma membrane in a locally concentrated manner in IAV-infected cells [155]. PS was observed to cluster, even in non-infected cells. Therefore, in these experiments, the fluorescent M1 derivative might have been targeted to pre-existing PS clusters. At this time, it remains to be determined whether M1 multimerization restricts the lateral diffusion of PS at the plasma membrane, thereby stabilizing the PS-enriched domain. Additionally, the effects of M1 in cells were not examined in the absence of other viral components, leaving the possible roles for other IAV proteins (e.g., HA and M2) in the M1-PS coclustering open.

Even though the effects of M2 on membrane curvature have been intensely studied, studies conducted *in vitro* using giant unilamellar vesicles (GUVs) have shown that M1 can also cause membrane curvature in the presence of acidic phospholipids [151,156]. This process is reportedly driven by charge rather than by lipid headgroup specificity, since PS, PG, and PI(4,5)P2 were all described to support the M1-dependent membrane deformation [151,156].

X-ray crystallography and cryo-electron tomography studies determined that the N-terminal domain of M1 protein contains a highly positively charged surface [144,157] (Figure 1a). The three arginine residues spanning the positions 76–78 in M1 are essential for M1 membrane binding and virus assembly [144,149]. Replacing the basic residues with neutral amino acids causes mislocalization of the M1 proteins in cells [149] and a defect in binding to liposomes containing acidic phospholipids, whether PS or PI(4,5)P2 [146]. Of note, recent studies have shown that M1 also interacts with PI(4,5)P2 in cells. Single molecule localization microscopy experiments demonstrated that M1 co-clusters with PI(4,5)P2; however, this co-clustering can be undone by incubating the cells with positively charged compounds like CPC [137]. Notably, co-clustering between M1 and HA at the plasma membrane can be disrupted by CPC, indicating that the PI(4,5)P2 clustering is important for the association between M1 and HA at the plasma membrane [137] (Figure 2b).

In addition to HA and M1, the nucleoprotein (NP), an essential component of vRNPs, was shown to bind to PI(4,5)P2 using *in vitro* binding assays. Additionally, the depletion of cellular PI(4,5)P2 was observed to prevent the binding of NP to the plasma membrane in both cells transiently expressing NP alone and cells infected with IAV [158]. Altogether, the studies outlined above have demonstrated that acidic phospholipids regulate multiple steps in IAV particle formation, including the proper distribution of M1 and NP (or potentially, vRNP) to the plasma membrane, M1 multimerization, HA-M1 co-clustering, and the generation of membrane curvature, thereby facilitating the efficient replication cycles.

5. Human Immunodeficiency Virus Type 1 (HIV-1) Assembly and Acidic Phospholipids

5.1. HIV-1 Assembly

In general, HIV-1 assembly takes place at the plasma membrane of infected cells, where the polyprotein Gag orchestrates the virus assembly and budding [18–20]. Gag is translated as a precursor polyprotein Pr55Gag, consisting of the matrix domain (MA), the capsid domain (CA), spacer peptide 1 (SP1), the nucleocapsid domain (NC), spacer peptide 2 (SP2), and the p6 domain (Figure 1b) [18]. The MA domain contains two signals that allow for Gag localization to the plasma membrane. First, MA receives a co-translational modification

with a 14-carbon fatty acid, i.e., myristylation, on its N-terminal glycine, which provides MA with the capacity to interact with membranes via hydrophobic interactions [159–168]. Second, MA contains a conserved highly basic region (HBR), comprised of the positively charged amino acids lysine and arginine, which mediates Gag binding to the negatively charged phospholipid [18,161,162,169,170], in particular, PI(4,5)P₂, as discussed below (Figure 1b). At the plasma membrane, Gag undergoes multimerization mediated by CA–CA and NC–RNA interactions [18]. The specific interactions of NC with full-length viral RNA is primarily responsible for genome packaging, while MA plays an important role in the incorporation of the HIV-1 envelope protein (Env) [18]. The p6 domain of Gag interacts with the ESCRT machinery to mediate the pinching off event [18]. After the particle release, Gag undergoes a series of viral protease-mediated cleavages, leading to maturation of the infectious particle [18,171].

5.2. The Interplay Between Cellular Phospholipids and HIV-1 Structural Protein Gag

5.2.1. Gag and PI(4,5)P₂

PI(4,5)P₂ plays a pivotal role in HIV-1 assembly [18,172]. The depletion of cellular PI(4,5)P₂, achieved by the expression of polyphosphoinositide 5-phosphatase IV, significantly impairs Gag localization to the plasma membrane, as well as particle assembly [173–178]. Moreover, the overexpression of a constitutively active Arf6, which leads to PI(4,5)P₂ accumulation to the intracellular vesicles, redirects HIV-1 assembly and budding to these structures [173]. In addition, the inhibition of the Rab27-dependent trafficking of a kinase producing PI(4,5)P₂ precursor to the plasma membrane [179], as well as knockdown of PI(4,5)P₂-producing kinases [180], inhibits Gag localization to the plasma membrane. Altogether, these studies demonstrate the importance of PI(4,5)P₂ in determining the location of HIV-1 assembly [172]. As for the interface for PI(4,5)P₂ in Gag, genetic studies have revealed that the MA HBR of Gag is crucial for its interaction with PI(4,5)P₂. Substitutions of basic residues within the HBR with neutral amino acids result in the mislocalization of Gag to the intracellular compartments and/or failure to bind any membranes [162,169,170,181,182]. Liposome binding and NMR experiments showed that the same HBR basic amino acid residues are important for PI(4,5)P₂ interactions [178,182–185].

Although other phospholipids also contain negative charges on their phosphorylated headgroups, as it is the case for PS, *in vitro* lipid binding studies showed that the MA or Gag preferentially binds to PI(4,5)P₂ in a manner that is not simply charge-dependent [178,186–189]. NMR-based studies also demonstrate that MA has a higher affinity for PI(4,5)P₂ than for other phosphoinositides [183,190]. One NMR study using myristylated MA and a water-soluble PI(4,5)P₂ analogue detected sequestration of the short 2' acyl chain into the MA hydrophobic cleft [190], but this may be due to the use of the lipid with non-native short acyl chains [183]. A recent cryo-electron tomography study found that the mature Gag lattice showed a density consistent with the acyl chain sequestration of PI(4,5)P₂ [191], although the identity of this density remains to be determined. Nonetheless, PI(4,5)P₂ acyl chains, or rather, their saturation status, have been observed to affect Gag binding to lipid membranes [192].

Intriguingly, Gag MA has been shown to bind not only to PI(4,5)P₂ but also to nucleic acids [193–198], and this MA–nucleic acid interaction can compete with MA's binding to acidic lipids such as PS [182,199,200]. More specifically, *in vitro* liposome binding studies, including those performed in the presence of mammalian cell lysates, and cell-based Gag–RNA crosslinking studies collectively suggest that Gag utilizes cellular tRNAs to prevent MA HBR from binding to membranes that contain PS, which is ubiquitously present not only in the plasma membrane but also in the intracellular compartments [181,182,201–207]. Consistent with this notion, the interface in MA for tRNA largely overlaps with that for acidic phospholipids [208–210]. The current working model suggests that upon encountering PI(4,5)P₂ at the plasma membrane, Gag replaces the MA HBR-bound tRNA with this lipid, thereby ensuring the localization to the plasma membrane [172]. Additionally, it is

postulated that this mechanism may mediate the temporal regulation of Gag membrane binding [205,210].

Coarse-grained and long-timescale AAMD studies have shown that Gag and PI(4,5)P2 localize to the vicinity of each other in the membrane [211,212]. Moreover, stimulated emission depletion and fluorescence correlation spectroscopy have revealed that Gag can trap PI(4,5)P2 and cholesterol, but not phosphatidylethanolamine and sphingomyelin, around itself [213]. Lipidomics studies have demonstrated that the HIV-1 particle is enriched with PI(4,5)P2 compared to the composition of the plasma membrane of the producer cell [174,214]. These studies indicate that HIV assembly causes the relocalization and accumulation of PI(4,5)P2 to the particle assembly site in the plasma membrane. Due to its ability to multimerize via the CA and NC domains, membrane-bound Gag may form a lattice with large patches of basic residues facing the cytoplasmic leaflet of the plasma membrane, potentially promoting the enrichment or clustering of anionic lipids. In support of Gag multimer-dependent PI(4,5)P2 sequestration, *in vitro* studies using model membranes have demonstrated that Gag can induce the accumulation of PI(4,5)P2 around itself. Importantly, the highest accumulation of PI(4,5)P2 was observed when a full-length Gag was used, highlighting the contribution of multimerization to PI(4,5)P2 accumulation [215,216]. Interestingly, Gag also showed some preference for binding to pre-formed clusters of PI(4,5)P2 induced by cations like Ca²⁺ in studies performed in liposomes [215]. These results highlight Gag's versatility, as it can both cause PI(4,5)P2 clustering and utilize pre-formed PI(4,5)P2 clusters, at least *in vitro*.

While PI(4,5)P2 enrichment in assembling and released virus particles is recognized (Figure 2c), whether or not it performs any physiological function remains unknown. Interestingly, TIRF STORM super-resolution studies performed in HeLa and T cells demonstrated that Gag HBR and the JMBPS of the host transmembrane proteins, CD43, PSGL-1, and CD44, are important for their co-clustering at the assembly sites [217]. Importantly, these host proteins are incorporated into HIV-1 particles, and they can act as pro- or anti-viral factors, altering the fate of released virus particles [218–223]. In support of a role for PI(4,5)P2 in host protein incorporation, we observed that PI(4,5)P2 promotes Gag co-clustering with, and virus incorporation of, CD43, PSGL-1, and CD44 [224]. Together, these observations suggest that PI(4,5)P2 at the assembly sites plays a role not only in facilitating HIV-1 particle assembly but also regulating the sorting of host factors into the released particle.

5.2.2. HIV-1 and PS

While PI(4,5)P2 promotes HIV-1 assembly by interacting with Gag, the role of PS seems to be less clear. Liposome binding studies showed that the MA domain in Gag can bind to membranes containing PS [161,225]. An NMR study suggested that MA interacts simultaneously with PI(4,5)P2 and PS [226], although the latter apparently binds to a region outside of the HBR. An AAMD study using the membrane models containing both PI(4,5)P2 and PS showed that both anionic lipids interact with MA around the HBR [227]. However, as alluded to earlier, in the presence of cell lysates, this binding of Gag to PS is inhibited by the presence of RNA [228]. Therefore, the precise role PS plays in Gag localization to the plasma membrane remains to be determined.

Many viral infections, including HIV-1 infection, promote the exposure of PS to the outer leaflet of the infected cells [229,230]. One mechanism for this PS exposure on the host cell membrane is an apoptotic response induced by HIV-1. As a consequence, during the assembly, the virus incorporates this exposed PS into its particles [231]. Once incorporated into the particle envelope, PS appears to play a dual role in HIV-1 dissemination [229]. PS exposure on the outer membrane acts as an “eat me” signal [232,233], a mechanism exploited by the vaccinia virus [234,235], which facilitates contact with and internalization by potential target cells, contributing to viral spread. Likewise, PS exposure on the envelope facilitates HIV-1 infection by increasing virus binding to PS receptors on the target cell. Therefore, in this context, PS serves as a co-factor aiding in viral spread [231]. However, PS

can also promote cellular antiviral activities. HIV-1 particles exposing PS can be trapped by the TIM family proteins expressed in virus producer cells [236] and hence, fail to spread to target cells. PS is also implicated in the antiviral function of a host multi-transmembrane protein, SERINC5 [237,238]. SERINC5 is incorporated into virions and acts as a restriction factor, primarily by altering Env conformation and suppressing its activity [239]. Although the precise mechanism by which SERINC5 impairs viral infectivity is still to be determined, several studies have shown that SERINC5 modulates PS on the viral envelope [239]. A recent study demonstrated that SERINC5 has the ability to disrupt the membrane asymmetry of the HIV-1 envelope by exposing PS, PC, and PE on the outer leaflet, and that the degree of PS exposure correlates with the reduction in infectivity [240]. A subsequent study confirmed the phospholipid scramblase-like activity of SERINC5, but observed that the externalized PS levels do not strictly correlate with the negative impact of SERINC5 on viral infectivity [241]. Altogether, these findings suggest that PS in the HIV-1 particles, especially in the outer leaflet of the envelope membrane, may prevent or facilitate HIV-1 infectivity and spread; however, the precise role played by PS in HIV-1 assembly and spread remains to be determined.

6. Future Directions

The studies on IAV and HIV-1 outlined above collectively support the pivotal roles played by the plasma membrane acidic phospholipids in assembly and budding. The interaction between the structural proteins of these enveloped viruses and acidic phospholipids affects the steps of the virus particle assembly process, such as the binding of the proteins to the plasma membrane, oligomerization of the proteins, and generation of membrane curvature. During this process, the viral structural proteins can dramatically alter the local organization of the plasma membrane. It is also likely that multiple lipids temporally or stably participate in promoting protein clustering, creating a conducive environment for viral assembly and budding.

Although the recent studies have advanced our understanding of the relationships between acidic phospholipids and IAV or HIV-1, several questions, which are often common regarding both viruses, remain to be addressed. The significance of these questions is not limited to the two viruses, since the assembly processes of other enveloped viruses, including filoviruses [40–45] and paramyxoviruses [46,242,243], which comprise major emerging or reemerging viruses, are now known to involve acidic phospholipids. We highlight several of the outstanding questions below.

- (1) Are viral structural proteins recruited to pre-existing acidic phospholipid-rich areas, or do they cause acidic phospholipids clustering or both?

Biophysical and cell-based studies have shown that host cellular proteins, such as MARCKS [66,68,244], syntaxin-1 [90], and other proteins with positively charged sequences (Figure 2a,d), induce the clustering of acidic phospholipids around them. Therefore, it is likely that acidic phospholipid clusters pre-exist at the plasma membrane before viral structural proteins are expressed. However, it is unknown if these clusters serve as the site of virus assembly (Figure 2d).

In the case of HIV-1, the reduction of the PI(4,5)P2 diffusion rate at the Gag clusters and PI(4,5)P2 enrichment in the virions support the possibility that viral structural proteins can generate acidic phospholipid clusters at the assembly sites (Figure 2c). However, it remains to be determined whether the acidic phospholipid clusters formed at the assembly sites are stable in the time scale of the assembly process (i.e., minutes to tens of minutes) and whether these clusters have any function (see below).

- (2) Do acidic phospholipids play a role in the incorporation of viral transmembrane proteins and the packaging of viral genomes into nascent virus particles?

The studies described above revealed that acidic phospholipids, in particular PI(4,5)P2, associate with both HA and M1 and promote HA-M1 co-clustering [128,137], which may facilitate the formation of the virus assembly sites (Figure 2b). Therefore, it is likely that

PI(4,5)P2 promotes the incorporation of HA into nascent IAV particles. However, the mechanistic details remain to be determined. As for HIV-1, it remains to be determined whether acidic phospholipids are involved in the incorporation of viral glycoprotein Env into assembling virus particles, even though a polybasic surface has been identified in the Env cytoplasmic tail [245].

HIV-1 Gag binds both plasma membrane and viral genomic RNA and is therefore solely responsible for genome packaging during the process of particle assembly. In nascent IAV particles, however, vRNP complexes are bound to the M1 lattice but not to the lipid bilayer [246,247]. Therefore, even though PI(4,5)P2 was shown to be important for the plasma membrane localization of NP and genome packaging into virus particles [158], it remains unknown whether or how NP or vRNP switch the binding partner from PI(4,5)P2 to M1.

- (3) Do acidic phospholipids regulate the recruitment of host cellular proteins to the assembly sites, and if so, what roles do these host proteins play in the assembly process or virion infectivity?

Our study has shown that the incorporation of CD43, PSGL-1, and CD44, which contain juxtamembrane polybasic sequences, into HIV particles was reduced up to 20-fold upon PI(4,5)P2 depletion [224], indicating that PI(4,5)P2 promotes the association between these host cellular transmembrane proteins and HIV-1 Gag (Figure 2c,d). It is conceivable that the mechanism by which these host transmembrane proteins are incorporated into HIV-1 particles may be analogous to the association between IAV M1 and HA at the plasma membrane during assembly (Figure 2b). If the acidic phospholipids attract host transmembrane proteins solely via electrostatic interactions, it is crucial to understand whether and how the viruses regulate the recruitment of a variety of transmembrane proteins with basic cytoplasmic sequences to the assembly sites, since these host transmembrane proteins could have pro-viral (e.g., CD44) or anti-viral (e.g., CD43 and PSGL-1) effects on virus infectivity or spread.

In addition to recruiting the host transmembrane proteins, the clustering of acidic phospholipids, in particular PI(4,5)P2, at the virus assembly sites, may recruit other host proteins that may facilitate or suppress specific steps in the virus assembly process (Figure 2c). For example, some membrane-modulating proteins (e.g., IRSp53 [248]) and the actin cytoskeleton are regulated by PI(4,5)P2 and have been shown to affect the virus assembly process [13,249,250].

- (4) What is the role, if any, of the incorporated acidic phospholipids for IAV and HIV in viral spread?

The accumulation of acidic phospholipids at the assembly sites likely leads to the presence, if not enrichment, of the lipids in the nascent virus particles. While the studies discussed in this review and elsewhere [229,251,252] support the roles for PS incorporated into enveloped virus particles, the roles played by virion-associated PI(4,5)P2 during the early stages of the virus replication cycle, i.e., attachment and entry, are poorly understood. Notably, cryo-EM studies revealed that the maturation of the HIV-1 virions apparently changes the patterns of interactions between MA and PI(4,5)P2 [191], which led the authors to propose their possible signaling function upon fusion with the target cell membrane.

- (5) How do viral proteins regulate acidic phospholipid distribution locally and globally?

To address the questions above, it will be essential to determine the molecular interfaces in the viral structural proteins and the contribution of their oligomerization to the acidic phospholipid distribution. In this review, we focused on the viral structural proteins, HA, M1, and Gag; however, it will be also important to examine whether other viral proteins play any roles, e.g., by locally or globally modulating cellular enzymes that favor the accumulation of acidic phospholipids at the viral assembly sites.

The questions listed above highlight some, but not all, areas in which further research is needed. The advancement of research methodologies including, but not limited to,

structural, microscopic, and omics approaches, will allow for our deeper understanding of the intricate interplay between enveloped viruses and acidic phospholipids during infection, potentially illuminating new therapeutic strategies for controlling enveloped virus assembly and spread.

Author Contributions: Writing—original draft preparation, R.d.S.C. and A.O.; writing—review and editing, R.d.S.C. and A.O.; funding acquisition, A.O. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by NIH grant R37 AI 071727 (to A.O). The APC was funded by the same grant.

Acknowledgments: We thank the Ono lab members for the active discussion and valuable suggestions.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Welsch, S.; Miller, S.; Romero-Brey, I.; Merz, A.; Bleck, C.K.; Walther, P.; Fuller, S.D.; Antony, C.; Krijnse-Locker, J.; Bartenschlager, R. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe* **2009**, *5*, 365–375. [[CrossRef](#)] [[PubMed](#)]
- Mackenzie, J.M.; Jones, M.K.; Young, P.R. Immunolocalization of the dengue virus nonstructural glycoprotein NS1 suggests a role in viral RNA replication. *Virology* **1996**, *220*, 232–240. [[CrossRef](#)] [[PubMed](#)]
- Barnard, T.R.; Abram, Q.H.; Lin, Q.F.; Wang, A.B.; Sagan, S.M. Molecular Determinants of Flavivirus Virion Assembly. *Trends Biochem. Sci.* **2021**, *46*, 378–390. [[CrossRef](#)]
- Barbosa, N.S.; Mendonca, L.R.; Dias, M.V.S.; Pontelli, M.C.; da Silva, E.Z.M.; Criado, M.F.; da Silva-Januario, M.E.; Schindler, M.; Jamur, M.C.; Oliver, C.; et al. ESCRT machinery components are required for Orthobunyavirus particle production in Golgi compartments. *PLoS Pathog.* **2018**, *14*, e1007047. [[CrossRef](#)] [[PubMed](#)]
- Barker, J.; daSilva, L.L.P.; Crump, C.M. Mechanisms of bunyavirus morphogenesis and egress. *J. Gen. Virol.* **2023**, *104*, 001845. [[CrossRef](#)] [[PubMed](#)]
- Draganova, E.B.; Valentin, J.; Heldwein, E.E. The Ins and Outs of Herpesviral Capsids: Divergent Structures and Assembly Mechanisms across the Three Subfamilies. *Viruses* **2021**, *13*, 1913. [[CrossRef](#)]
- Selzer, L.; Zlotnick, A. Assembly and Release of Hepatitis B Virus. *Cold Spring Harb. Perspect. Med.* **2015**, *5*, a021394. [[CrossRef](#)]
- Roingard, P.; Eymieux, S.; Burlaud-Gaillard, J.; Hourieux, C.; Patient, R.; Blanchard, E. The double-membrane vesicle (DMV): A virus-induced organelle dedicated to the replication of SARS-CoV-2 and other positive-sense single-stranded RNA viruses. *Cell. Mol. Life Sci.* **2022**, *79*, 425. [[CrossRef](#)]
- Roberts, S.R.; Compans, R.W.; Wertz, G.W. Respiratory syncytial virus matures at the apical surfaces of polarized epithelial cells. *J. Virol.* **1995**, *69*, 2667–2673. [[CrossRef](#)]
- Cardoso, R.S.; Tavares, L.A.; Jesus, B.L.S.; Criado, M.F.; de Carvalho, A.N.; Souza, J.P.; Bedi, S.; de Souza, M.M.; Silva, M.L.; Lanfredi, G.P.; et al. Host Retromer Protein Sorting Nexin 2 Interacts with Human Respiratory Syncytial Virus Structural Proteins and is Required for Efficient Viral Production. *mBio* **2020**, *11*, e01869-20. [[CrossRef](#)]
- Sugrue, R.J.; Tan, B.H. Defining the Assembleome of the Respiratory Syncytial Virus. In *Virus Infected Cells; Subcellular Biochemistry*; Springer: Berlin/Heidelberg, Germany, 2023; Volume 106, pp. 227–249.
- Rossman, J.S.; Lamb, R.A. Influenza virus assembly and budding. *Virology* **2011**, *411*, 229–236. [[CrossRef](#)] [[PubMed](#)]
- Bedi, S.; Noda, T.; Kawaoka, Y.; Ono, A. A Defect in Influenza A Virus Particle Assembly Specific to Primary Human Macrophages. *mBio* **2018**, *9*, e01916-18. [[CrossRef](#)]
- Nayak, D.P.; Balogun, R.A.; Yamada, H.; Zhou, Z.H.; Barman, S. Influenza virus morphogenesis and budding. *Virus Res.* **2009**, *143*, 147–161. [[CrossRef](#)] [[PubMed](#)]
- Geisbert, T.W.; Jahrling, P.B. Differentiation of filoviruses by electron microscopy. *Virus Res.* **1995**, *39*, 129–150. [[CrossRef](#)] [[PubMed](#)]
- Noda, T.; Ebihara, H.; Muramoto, Y.; Fujii, K.; Takada, A.; Sagara, H.; Kim, J.H.; Kida, H.; Feldmann, H.; Kawaoka, Y. Assembly and budding of Ebolavirus. *PLoS Pathog.* **2006**, *2*, e99. [[CrossRef](#)]
- Dolnik, O.; Becker, S. Assembly and transport of filovirus nucleocapsids. *PLoS Pathog.* **2022**, *18*, e1010616. [[CrossRef](#)]
- Freed, E.O. HIV-1 assembly, release and maturation. *Nat. Rev. Microbiol.* **2015**, *13*, 484–496. [[CrossRef](#)]
- Jouvenet, N.; Neil, S.J.; Bess, C.; Johnson, M.C.; Virgen, C.A.; Simon, S.M.; Bieniasz, P.D. Plasma membrane is the site of productive HIV-1 particle assembly. *PLoS Biol.* **2006**, *4*, e435. [[CrossRef](#)]
- Finzi, A.; Orthwein, A.; Mercier, J.; Cohen, E.A. Productive human immunodeficiency virus type 1 assembly takes place at the plasma membrane. *J. Virol.* **2007**, *81*, 7476–7490. [[CrossRef](#)]
- Fahy, E.; Subramaniam, S.; Murphy, R.C.; Nishijima, M.; Raetz, C.R.; Shimizu, T.; Spener, F.; van Meer, G.; Wakelam, M.J.; Dennis, E.A. Update of the LIPID MAPS comprehensive classification system for lipids. *J. Lipid Res.* **2009**, *50*, S9–S14. [[CrossRef](#)]

22. Harayama, T.; Riezman, H. Understanding the diversity of membrane lipid composition. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 281–296. [[CrossRef](#)]
23. IUPAC-IUB Commission on Biochemical Nomenclature. Nomenclature of phosphorus-containing compounds of biochemical importance (Recommendations 1976). *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 2222–2230. [[CrossRef](#)] [[PubMed](#)]
24. Vance, J.E. Historical perspective: Phosphatidylserine and phosphatidylethanolamine from the 1800s to the present. *J. Lipid Res.* **2018**, *59*, 923–944. [[CrossRef](#)] [[PubMed](#)]
25. Posor, Y.; Jang, W.; Haucke, V. Phosphoinositides as membrane organizers. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 797–816. [[CrossRef](#)] [[PubMed](#)]
26. Kay, J.G.; Fairn, G.D. Distribution, dynamics and functional roles of phosphatidylserine within the cell. *Cell Commun. Signal.* **2019**, *17*, 126. [[CrossRef](#)]
27. van Meer, G.; Voelker, D.R.; Feigenson, G.W. Membrane lipids: Where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 112–124. [[CrossRef](#)]
28. Lolicato, F.; Nickel, W.; Haucke, V.; Ebner, M. Phosphoinositide switches in cell physiology—From molecular mechanisms to disease. *J. Biol. Chem.* **2024**, *300*, 105757. [[CrossRef](#)]
29. Nicolson, G.L. The Fluid-Mosaic Model of Membrane Structure: Still relevant to understanding the structure, function and dynamics of biological membranes after more than 40 years. *Biochim. Biophys. Acta* **2014**, *1838*, 1451–1466. [[CrossRef](#)]
30. Sakuragi, T.; Nagata, S. Regulation of phospholipid distribution in the lipid bilayer by flippases and scramblases. *Nat. Rev. Mol. Cell Biol.* **2023**, *24*, 576–596. [[CrossRef](#)]
31. Kervin, T.A.; Overduin, M. Membranes are functionalized by a proteolipid code. *BMC Biol.* **2024**, *22*, 46. [[CrossRef](#)]
32. Thakur, R.; Naik, A.; Panda, A.; Raghu, P. Regulation of Membrane Turnover by Phosphatidic Acid: Cellular Functions and Disease Implications. *Front. Cell Dev. Biol.* **2019**, *7*, 83. [[CrossRef](#)] [[PubMed](#)]
33. McLaughlin, S.; Murray, D. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* **2005**, *438*, 605–611. [[CrossRef](#)] [[PubMed](#)]
34. Shin, J.J.H.; Loewen, C.J.R. Putting the pH into phosphatidic acid signaling. *BMC Biol.* **2011**, *9*, 85. [[CrossRef](#)] [[PubMed](#)]
35. Hirama, T.; Lu, S.M.; Kay, J.G.; Maekawa, M.; Kozlov, M.M.; Grinstein, S.; Fairn, G.D. Membrane curvature induced by proximity of anionic phospholipids can initiate endocytosis. *Nat. Commun.* **2017**, *8*, 1393. [[CrossRef](#)]
36. Bills, B.L.; Knowles, M.K. Phosphatidic Acid Accumulates at Areas of Curvature in Tubulated Lipid Bilayers and Liposomes. *Biomolecules* **2022**, *12*, 1707. [[CrossRef](#)]
37. Gericke, A. Is Calcium Fine-Tuning Phosphoinositide-Mediated Signaling Events Through Clustering? *Biophys. J.* **2018**, *114*, 2483–2484. [[CrossRef](#)]
38. Schink, K.O.; Tan, K.W.; Stenmark, H. Phosphoinositides in Control of Membrane Dynamics. *Annu. Rev. Cell Dev. Biol.* **2016**, *32*, 143–171. [[CrossRef](#)]
39. Beziau, A.; Brand, D.; Piver, E. The Role of Phosphatidylinositol Phosphate Kinases during Viral Infection. *Viruses* **2020**, *12*, 1124. [[CrossRef](#)]
40. Adu-Gyamfi, E.; Johnson, K.A.; Fraser, M.E.; Scott, J.L.; Soni, S.P.; Jones, K.R.; Digman, M.A.; Gratton, E.; Tessier, C.R.; Stahelin, R.V. Host Cell Plasma Membrane Phosphatidylserine Regulates the Assembly and Budding of Ebola Virus. *J. Virol.* **2015**, *89*, 9440–9453. [[CrossRef](#)]
41. Gc, J.B.; Gerstman, B.S.; Stahelin, R.V.; Chapagain, P.P. The Ebola virus protein VP40 hexamer enhances the clustering of PI(4,5)P(2) lipids in the plasma membrane. *Phys. Chem. Chem. Phys.* **2016**, *18*, 28409–28417. [[CrossRef](#)]
42. Johnson, K.A.; Taghon, G.J.; Scott, J.L.; Stahelin, R.V. The Ebola Virus matrix protein, VP40, requires phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) for extensive oligomerization at the plasma membrane and viral egress. *Sci. Rep.* **2016**, *6*, 19125. [[CrossRef](#)] [[PubMed](#)]
43. Acciani, M.D.; Lay Mendoza, M.F.; Havranek, K.E.; Duncan, A.M.; Iyer, H.; Linn, O.L.; Brindley, M.A. Ebola Virus Requires Phosphatidylserine Scrambling Activity for Efficient Budding and Optimal Infectivity. *J. Virol.* **2021**, *95*, e0116521. [[CrossRef](#)] [[PubMed](#)]
44. Cioffi, M.D.; Husby, M.L.; Gerstman, B.S.; Stahelin, R.V.; Chapagain, P.P. Role of phosphatidic acid lipids on plasma membrane association of the Ebola virus matrix protein VP40. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2024**, *1869*, 159464. [[CrossRef](#)] [[PubMed](#)]
45. Johnson, K.A.; Budicini, M.R.; Bhattarai, N.; Sharma, T.; Urata, S.; Gerstman, B.S.; Chapagain, P.P.; Li, S.; Stahelin, R.V. PI(4,5)P(2) binding sites in the Ebola virus matrix protein VP40 modulate assembly and budding. *J. Lipid Res.* **2024**, *65*, 100512. [[CrossRef](#)] [[PubMed](#)]
46. Norris, M.J.; Husby, M.L.; Kiosses, W.B.; Yin, J.; Saxena, R.; Rennick, L.J.; Heiner, A.; Harkins, S.S.; Pokhrel, R.; Schendel, S.L.; et al. Measles and Nipah virus assembly: Specific lipid binding drives matrix polymerization. *Sci. Adv.* **2022**, *8*, eabn1440. [[CrossRef](#)]
47. Eisenberg, S.; Haimov, E.; Walpole, G.F.W.; Plumb, J.; Kozlov, M.M.; Grinstein, S. Mapping the electrostatic profiles of cellular membranes. *Mol. Biol. Cell* **2021**, *32*, 301–310. [[CrossRef](#)]
48. Yeung, T.; Gilbert, G.E.; Shi, J.; Silvius, J.; Kapus, A.; Grinstein, S. Membrane phosphatidylserine regulates surface charge and protein localization. *Science* **2008**, *319*, 210–213. [[CrossRef](#)]
49. McLaughlin, S. The electrostatic properties of membranes. *Annu. Rev. Biophys. Biophys. Chem.* **1989**, *18*, 113–136. [[CrossRef](#)]

50. Raghupathy, R.; Anilkumar, A.A.; Polley, A.; Singh, P.P.; Yadav, M.; Johnson, C.; Suryawanshi, S.; Saikam, V.; Sawant, S.D.; Panda, A.; et al. Transbilayer lipid interactions mediate nanoclustering of lipid-anchored proteins. *Cell* **2015**, *161*, 581–594. [[CrossRef](#)]
51. Leventis, P.A.; Grinstein, S. The distribution and function of phosphatidylserine in cellular membranes. *Annu. Rev. Biophys.* **2010**, *39*, 407–427. [[CrossRef](#)]
52. Varga, K.; Jiang, Z.J.; Gong, L.W. Phosphatidylserine is critical for vesicle fission during clathrin-mediated endocytosis. *J. Neurochem.* **2020**, *152*, 48–60. [[CrossRef](#)] [[PubMed](#)]
53. Hallett, M.B. Localisation of Intracellular Signals and Responses during Phagocytosis. *Int. J. Mol. Sci.* **2023**, *24*, 2825. [[CrossRef](#)]
54. Bohdanowicz, M.; Grinstein, S. Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis. *Physiol. Rev.* **2013**, *93*, 69–106. [[CrossRef](#)]
55. Uchida, Y.; Hasegawa, J.; Chinnapen, D.; Inoue, T.; Okazaki, S.; Kato, R.; Wakatsuki, S.; Misaki, R.; Koike, M.; Uchiyama, Y.; et al. Intracellular phosphatidylserine is essential for retrograde membrane traffic through endosomes. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 15846–15851. [[CrossRef](#)]
56. Fairn, G.D.; Hermansson, M.; Somerharju, P.; Grinstein, S. Phosphatidylserine is polarized and required for proper Cdc42 localization and for development of cell polarity. *Nat. Cell Biol.* **2011**, *13*, 1424–1430. [[CrossRef](#)]
57. Ammar, M.R.; Kassas, N.; Chasserot-Golaz, S.; Bader, M.F.; Vitale, N. Lipids in Regulated Exocytosis: What are They Doing? *Front. Endocrinol.* **2013**, *4*, 125. [[CrossRef](#)]
58. McLaughlin, S.; Wang, J.; Gambhir, A.; Murray, D. PIP(2) and proteins: Interactions, organization, and information flow. *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 151–175. [[CrossRef](#)]
59. Katan, M.; Cockcroft, S. Phosphatidylinositol(4,5)bisphosphate: Diverse functions at the plasma membrane. *Essays Biochem.* **2020**, *64*, 513–531.
60. Jost, M.; Simpson, F.; Kavran, J.M.; Lemmon, M.A.; Schmid, S.L. Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation. *Curr. Biol.* **1998**, *8*, 1399–1402. [[CrossRef](#)]
61. Crul, T.; Maleth, J. Endoplasmic Reticulum-Plasma Membrane Contact Sites as an Organizing Principle for Compartmentalized Calcium and cAMP Signaling. *Int. J. Mol. Sci.* **2021**, *22*, 4703. [[CrossRef](#)]
62. Lahiri, S.; Toulmay, A.; Prinz, W.A. Membrane contact sites, gateways for lipid homeostasis. *Curr. Opin. Cell Biol.* **2015**, *33*, 82–87. [[CrossRef](#)] [[PubMed](#)]
63. Thallmair, V.; Schultz, L.; Evers, S.; Jolie, T.; Goecke, C.; Leitner, M.G.; Thallmair, S.; Oliver, D. Localization of the tubby domain, a PI(4,5)P2 biosensor, to E-Syt3-rich endoplasmic reticulum-plasma membrane junctions. *J. Cell Sci.* **2023**, *136*, jcs260848. [[CrossRef](#)] [[PubMed](#)]
64. Cockcroft, S.; Raghu, P. Phospholipid transport protein function at organelle contact sites. *Curr. Opin. Cell Biol.* **2018**, *53*, 52–60. [[CrossRef](#)]
65. Seki, K.; Sheu, F.S.; Huang, K.P. Binding of myristoylated alanine-rich protein kinase C substrate to phosphoinositides attenuates the phosphorylation by protein kinase C. *Arch. Biochem. Biophys.* **1996**, *326*, 193–201. [[CrossRef](#)]
66. Denisov, G.; Wanaski, S.; Luan, P.; Glaser, M.; McLaughlin, S. Binding of basic peptides to membranes produces lateral domains enriched in the acidic lipids phosphatidylserine and phosphatidylinositol 4,5-bisphosphate: An electrostatic model and experimental results. *Biophys. J.* **1998**, *74 Pt 1*, 731–744. [[CrossRef](#)]
67. Laux, T.; Fukami, K.; Thelen, M.; Golub, T.; Frey, D.; Caroni, P. GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. *J. Cell Biol.* **2000**, *149*, 1455–1472. [[CrossRef](#)]
68. Rauch, M.E.; Ferguson, C.G.; Prestwich, G.D.; Cafiso, D.S. Myristoylated alanine-rich C kinase substrate (MARCKS) sequesters spin-labeled phosphatidylinositol 4,5-bisphosphate in lipid bilayers. *J. Biol. Chem.* **2002**, *277*, 14068–14076. [[CrossRef](#)]
69. Pemberton, J.G.; Balla, T. Polyphosphoinositide-Binding Domains: Insights from Peripheral Membrane and Lipid-Transfer Proteins. *Adv. Exp. Med. Biol.* **2019**, *1111*, 77–137.
70. Wills, R.C.; Hammond, G.R.V. PI(4,5)P2: Signaling the plasma membrane. *Biochem. J.* **2022**, *479*, 2311–2325. [[CrossRef](#)]
71. Harlan, J.E.; Hajduk, P.J.; Yoon, H.S.; Fesik, S.W. Pleckstrin homology domains bind to phosphatidylinositol-4,5-bisphosphate. *Nature* **1994**, *371*, 168–170. [[CrossRef](#)]
72. Lomasney, J.W.; Cheng, H.F.; Wang, L.P.; Kuan, Y.; Liu, S.; Fesik, S.W.; King, K. Phosphatidylinositol 4,5-bisphosphate binding to the pleckstrin homology domain of phospholipase C-delta1 enhances enzyme activity. *J. Biol. Chem.* **1996**, *271*, 25316–25326. [[CrossRef](#)] [[PubMed](#)]
73. Lemmon, M.A. Pleckstrin homology (PH) domains and phosphoinositides. *Biochem. Soc. Symp.* **2007**, *74*, 81–93. [[CrossRef](#)] [[PubMed](#)]
74. Powis, G.; Meuillet, E.J.; Indarte, M.; Booher, G.; Kirkpatrick, L. Pleckstrin Homology [PH] domain, structure, mechanism, and contribution to human disease. *Biomed. Pharmacother.* **2023**, *165*, 115024. [[CrossRef](#)] [[PubMed](#)]
75. Singh, N.; Reyes-Ordóñez, A.; Compagnone, M.A.; Moreno, J.F.; Leslie, B.J.; Ha, T.; Chen, J. Redefining the specificity of phosphoinositide-binding by human PH domain-containing proteins. *Nat. Commun.* **2021**, *12*, 4339. [[CrossRef](#)]
76. Mattila, P.K.; Pykalainen, A.; Saarikangas, J.; Paavilainen, V.O.; Vihinen, H.; Jokitalo, E.; Lappalainen, P. Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism. *J. Cell Biol.* **2007**, *176*, 953–964. [[CrossRef](#)]
77. Ahmed, S.; Bu, W.; Lee, R.T.; Maurer-Stroh, S.; Goh, W.I. F-BAR domain proteins: Families and function. *Commun. Integr. Biol.* **2010**, *3*, 116–121. [[CrossRef](#)]

78. Itoh, T.; Koshiba, S.; Kigawa, T.; Kikuchi, A.; Yokoyama, S.; Takenawa, T. Role of the ENTH domain in phosphatidylinositol-4,5-bisphosphate binding and endocytosis. *Science* **2001**, *291*, 1047–1051. [[CrossRef](#)]
79. Ford, M.G.; Pearce, B.M.; Higgins, M.K.; Vallis, Y.; Owen, D.J.; Gibson, A.; Hopkins, C.R.; Evans, P.R.; McMahon, H.T. Simultaneous binding of PtdIns(4,5)P₂ and clathrin by AP180 in the nucleation of clathrin lattices on membranes. *Science* **2001**, *291*, 1051–1055. [[CrossRef](#)]
80. Roux, A.; Koster, G.; Lenz, M.; Sorre, B.; Manneville, J.B.; Nassoy, P.; Bassereau, P. Membrane curvature controls dynamin polymerization. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4141–4146. [[CrossRef](#)]
81. Kadlecova, Z.; Spielman, S.J.; Loerke, D.; Mohanakrishnan, A.; Reed, D.K.; Schmid, S.L. Regulation of clathrin-mediated endocytosis by hierarchical allosteric activation of AP2. *J. Cell Biol.* **2017**, *216*, 167–179. [[CrossRef](#)]
82. Krauss, M.; Kukhtina, V.; Pechstein, A.; Haucke, V. Stimulation of phosphatidylinositol kinase type I-mediated phosphatidylinositol (4,5)-bisphosphate synthesis by AP-2 cargo complexes. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11934–11939. [[CrossRef](#)] [[PubMed](#)]
83. Mu, L.; Tu, Z.; Miao, L.; Ruan, H.; Kang, N.; Hei, Y.; Chen, J.; Wei, W.; Gong, F.; Wang, B.; et al. A phosphatidylinositol 4,5-bisphosphate redistribution-based sensing mechanism initiates a phagocytosis programing. *Nat. Commun.* **2018**, *9*, 4259. [[CrossRef](#)] [[PubMed](#)]
84. Flannagan, R.S.; Jaumouille, V.; Grinstein, S. The cell biology of phagocytosis. *Annu. Rev. Pathol.* **2012**, *7*, 61–98. [[CrossRef](#)] [[PubMed](#)]
85. Golebiewska, U.; Kay, J.G.; Masters, T.; Grinstein, S.; Im, W.; Pastor, R.W.; Scarlata, S.; McLaughlin, S. Evidence for a fence that impedes the diffusion of phosphatidylinositol 4,5-bisphosphate out of the forming phagosomes of macrophages. *Mol. Biol. Cell* **2011**, *22*, 3498–3507. [[CrossRef](#)]
86. Swanson, J.A. Shaping cups into phagosomes and macropinosomes. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 639–649. [[CrossRef](#)]
87. Yang, X.; Tu, W.; Gao, X.; Zhang, Q.; Guan, J.; Zhang, J. Functional regulation of syntaxin-1: An underlying mechanism mediating exocytosis in neuroendocrine cells. *Front. Endocrinol.* **2023**, *14*, 1096365. [[CrossRef](#)]
88. Liang, T.; Xie, L.; Chao, C.; Kang, Y.; Lin, X.; Qin, T.; Xie, H.; Feng, Z.P.; Gaisano, H.Y. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) modulates interaction of syntaxin-1A with sulfonylurea receptor 1 to regulate pancreatic beta-cell ATP-sensitive potassium channels. *J. Biol. Chem.* **2014**, *289*, 6028–6040. [[CrossRef](#)]
89. Lam, A.D.; Tryoen-Toth, P.; Tsai, B.; Vitale, N.; Stuenkel, E.L. SNARE-catalyzed fusion events are regulated by Syntaxin1A-lipid interactions. *Mol. Biol. Cell* **2008**, *19*, 485–497. [[CrossRef](#)]
90. Honigsmann, A.; van den Bogaart, G.; Iraheta, E.; Risselada, H.J.; Milovanovic, D.; Mueller, V.; Mullar, S.; Diederichsen, U.; Fasshauer, D.; Grubmuller, H.; et al. Phosphatidylinositol 4,5-bisphosphate clusters act as molecular beacons for vesicle recruitment. *Nat. Struct. Mol. Biol.* **2013**, *20*, 679–686. [[CrossRef](#)]
91. van den Bogaart, G.; Meyenberg, K.; Risselada, H.J.; Amin, H.; Willig, K.I.; Hubrich, B.E.; Dier, M.; Hell, S.W.; Grubmuller, H.; Diederichsen, U.; et al. Membrane protein sequestering by ionic protein-lipid interactions. *Nature* **2011**, *479*, 552–555. [[CrossRef](#)]
92. Hammond, G.R. Does PtdIns(4,5)P₂ concentrate so it can multi-task? *Biochem. Soc. Trans.* **2016**, *44*, 228–233. [[CrossRef](#)] [[PubMed](#)]
93. Han, K.; Kim, S.H.; Venable, R.M.; Pastor, R.W. Design principles of PI(4,5)P₂ clustering under protein-free conditions: Specific cation effects and calcium-potassium synergy. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2202647119. [[CrossRef](#)] [[PubMed](#)]
94. Golebiewska, U.; Nyako, M.; Woturski, W.; Zaitseva, I.; McLaughlin, S. Diffusion coefficient of fluorescent phosphatidylinositol 4,5-bisphosphate in the plasma membrane of cells. *Mol. Biol. Cell* **2008**, *19*, 1663–1669. [[CrossRef](#)]
95. Gambhir, A.; Hangyas-Mihalyne, G.; Zaitseva, I.; Cafiso, D.S.; Wang, J.; Murray, D.; Pentylala, S.N.; Smith, S.O.; McLaughlin, S. Electrostatic sequestration of PI(4,5)P₂ on phospholipid membranes by basic/aromatic regions of proteins. *Biophys. J.* **2004**, *86*, 2188–2207. [[CrossRef](#)] [[PubMed](#)]
96. Koldso, H.; Shorthouse, D.; Helie, J.; Sansom, M.S. Lipid clustering correlates with membrane curvature as revealed by molecular simulations of complex lipid bilayers. *PLoS Comput. Biol.* **2014**, *10*, e1003911. [[CrossRef](#)]
97. Dou, D.; Revol, R.; Ostbye, H.; Wang, H.; Daniels, R. Influenza A Virus Cell Entry, Replication, Virion Assembly and Movement. *Front. Immunol.* **2018**, *9*, 1581. [[CrossRef](#)]
98. Carter, T.; Iqbal, M. The Influenza A Virus Replication Cycle: A Comprehensive Review. *Viruses* **2024**, *16*, 316. [[CrossRef](#)]
99. Wu, N.C.; Wilson, I.A. Influenza Hemagglutinin Structures and Antibody Recognition. *Cold Spring Harb. Perspect. Med.* **2020**, *10*, a038778. [[CrossRef](#)]
100. Chlanda, P.; Mekhedov, E.; Waters, H.; Sodt, A.; Schwartz, C.; Nair, V.; Blank, P.S.; Zimmerberg, J. Palmitoylation Contributes to Membrane Curvature in Influenza A Virus Assembly and Hemagglutinin-Mediated Membrane Fusion. *J. Virol.* **2017**, *91*, e00947-17. [[CrossRef](#)]
101. McAuley, J.L.; Gilbertson, B.P.; Trifkovic, S.; Brown, L.E.; McKimm-Breschkin, J.L. Influenza Virus Neuraminidase Structure and Functions. *Front. Microbiol.* **2019**, *10*, 39. [[CrossRef](#)]
102. Martin, K.; Helenius, A. Nuclear transport of influenza virus ribonucleoproteins: The viral matrix protein (M1) promotes export and inhibits import. *Cell* **1991**, *67*, 117–130. [[CrossRef](#)] [[PubMed](#)]
103. Li, X.; Gu, M.; Zheng, Q.; Gao, R.; Liu, X. Packaging signal of influenza A virus. *Virol. J.* **2021**, *18*, 36. [[CrossRef](#)] [[PubMed](#)]
104. Petrich, A.; Dunsing, V.; Bobone, S.; Chiantia, S. Influenza A M2 recruits M1 to the plasma membrane: A fluorescence fluctuation microscopy study. *Biophys. J.* **2021**, *120*, 5478–5490. [[CrossRef](#)] [[PubMed](#)]

105. Rossman, J.S.; Jing, X.; Leser, G.P.; Lamb, R.A. Influenza virus M2 protein mediates ESCRT-independent membrane scission. *Cell* **2010**, *142*, 902–913. [[CrossRef](#)]
106. Tam, V.C.; Quehenberger, O.; Oshansky, C.M.; Suen, R.; Armando, A.M.; Treuting, P.M.; Thomas, P.G.; Dennis, E.A.; Aderem, A. Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* **2013**, *154*, 213–227. [[CrossRef](#)]
107. Lin, S.; Liu, N.; Yang, Z.; Song, W.; Wang, P.; Chen, H.; Lucio, M.; Schmitt-Kopplin, P.; Chen, G.; Cai, Z. GC/MS-based metabolomics reveals fatty acid biosynthesis and cholesterol metabolism in cell lines infected with influenza A virus. *Talanta* **2010**, *83*, 262–268. [[CrossRef](#)]
108. Ivanova, P.T.; Myers, D.S.; Milne, S.B.; McClaren, J.L.; Thomas, P.G.; Brown, H.A. Lipid composition of viral envelope of three strains of influenza virus—Not all viruses are created equal. *ACS Infect. Dis.* **2015**, *1*, 399–452. [[CrossRef](#)]
109. Tanner, L.B.; Chng, C.; Guan, X.L.; Lei, Z.; Rozen, S.G.; Wenk, M.R. Lipidomics identifies a requirement for peroxisomal function during influenza virus replication. *J. Lipid Res.* **2014**, *55*, 1357–1365. [[CrossRef](#)]
110. Tisoncik-Go, J.; Gasper, D.J.; Kyle, J.E.; Eisfeld, A.J.; Selinger, C.; Hatta, M.; Morrison, J.; Korth, M.J.; Zink, E.M.; Kim, Y.M.; et al. Integrated Omics Analysis of Pathogenic Host Responses during Pandemic H1N1 Influenza Virus Infection: The Crucial Role of Lipid Metabolism. *Cell Host Microbe* **2016**, *19*, 254–266. [[CrossRef](#)]
111. Woods, P.S.; Doolittle, L.M.; Rosas, L.E.; Joseph, L.M.; Calomeni, E.P.; Davis, I.C. Lethal H1N1 influenza A virus infection alters the murine alveolar type II cell surfactant lipidome. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2016**, *311*, L1160–L1169. [[CrossRef](#)]
112. Schmitt, A.P.; Lamb, R.A. Influenza virus assembly and budding at the viral budzone. *Adv. Virus Res.* **2005**, *64*, 383–416. [[PubMed](#)]
113. Leser, G.P.; Lamb, R.A. Lateral Organization of Influenza Virus Proteins in the Budzone Region of the Plasma Membrane. *J. Virol.* **2017**, *91*, e02104-16. [[CrossRef](#)] [[PubMed](#)]
114. Li, Y.J.; Chen, C.Y.; Yang, J.H.; Chiu, Y.F. Modulating cholesterol-rich lipid rafts to disrupt influenza A virus infection. *Front. Immunol.* **2022**, *13*, 982264. [[CrossRef](#)] [[PubMed](#)]
115. Veit, M.; Engel, S.; Thaa, B.; Scolari, S.; Herrmann, A. Lipid domain association of influenza virus proteins detected by dynamic fluorescence microscopy techniques. *Cell. Microbiol.* **2013**, *15*, 179–189. [[CrossRef](#)]
116. Ono, A.; Freed, E.O. Role of lipid rafts in virus replication. *Adv. Virus Res.* **2005**, *64*, 311–358.
117. Zhang, J.; Pekosz, A.; Lamb, R.A. Influenza virus assembly and lipid raft microdomains: A role for the cytoplasmic tails of the spike glycoproteins. *J. Virol.* **2000**, *74*, 4634–4644. [[CrossRef](#)]
118. Skibbens, J.E.; Roth, M.G.; Matlin, K.S. Differential extractability of influenza virus hemagglutinin during intracellular transport in polarized epithelial cells and nonpolar fibroblasts. *J. Cell Biol.* **1989**, *108*, 821–832. [[CrossRef](#)]
119. Barman, S.; Nayak, D.P. Analysis of the transmembrane domain of influenza virus neuraminidase, a type II transmembrane glycoprotein, for apical sorting and raft association. *J. Virol.* **2000**, *74*, 6538–6545. [[CrossRef](#)]
120. Kundu, A.; Avalos, R.T.; Sanderson, C.M.; Nayak, D.P. Transmembrane domain of influenza virus neuraminidase, a type II protein, possesses an apical sorting signal in polarized MDCK cells. *J. Virol.* **1996**, *70*, 6508–6515. [[CrossRef](#)]
121. Ali, A.; Avalos, R.T.; Ponimaskin, E.; Nayak, D.P. Influenza virus assembly: Effect of influenza virus glycoproteins on the membrane association of M1 protein. *J. Virol.* **2000**, *74*, 8709–8719. [[CrossRef](#)]
122. Schroeder, C. Cholesterol-binding viral proteins in virus entry and morphogenesis. In *Cholesterol Binding and Cholesterol Transport Proteins; Subcellular Biochemistry*; Springer: Berlin/Heidelberg, Germany, 2010; Volume 51, pp. 77–108.
123. Leser, G.P.; Lamb, R.A. Influenza virus assembly and budding in raft-derived microdomains: A quantitative analysis of the surface distribution of HA, NA and M2 proteins. *Virology* **2005**, *342*, 215–227. [[CrossRef](#)] [[PubMed](#)]
124. Petrich, A.; Chiantia, S. Influenza A Virus Infection Alters Lipid Packing and Surface Electrostatic Potential of the Host Plasma Membrane. *Viruses* **2023**, *15*, 1830. [[CrossRef](#)] [[PubMed](#)]
125. Scheiffele, P.; Rietveld, A.; Wilk, T.; Simons, K. Influenza viruses select ordered lipid domains during budding from the plasma membrane. *J. Biol. Chem.* **1999**, *274*, 2038–2044. [[CrossRef](#)]
126. Veit, M.; Thaa, B. Association of influenza virus proteins with membrane rafts. *Adv. Virol.* **2011**, *2011*, 370606. [[CrossRef](#)] [[PubMed](#)]
127. Gerl, M.J.; Sampaio, J.L.; Urban, S.; Kalvodova, L.; Verbavatz, J.M.; Binnington, B.; Lindemann, D.; Lingwood, C.A.; Shevchenko, A.; Schroeder, C.; et al. Quantitative analysis of the lipidomes of the influenza virus envelope and MDCK cell apical membrane. *J. Cell Biol.* **2012**, *196*, 213–221. [[CrossRef](#)]
128. Raut, P.; Weller, S.R.; Obeng, B.; Soos, B.L.; West, B.E.; Potts, C.M.; Sangroula, S.; Kinney, M.S.; Burnell, J.E.; King, B.L.; et al. Cetylpyridinium chloride (CPC) reduces zebrafish mortality from influenza infection: Super-resolution microscopy reveals CPC interference with multiple protein interactions with phosphatidylinositol 4,5-bisphosphate in immune function. *Toxicol. Appl. Pharmacol.* **2022**, *440*, 115913. [[CrossRef](#)]
129. Hess, S.T.; Gould, T.J.; Gudheti, M.V.; Maas, S.A.; Mills, K.D.; Zimmerberg, J. Dynamic clustered distribution of hemagglutinin resolved at 40 nm in living cell membranes discriminates between raft theories. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 17370–17375. [[CrossRef](#)]
130. Hess, S.T.; Kumar, M.; Verma, A.; Farrington, J.; Kenworthy, A.; Zimmerberg, J. Quantitative electron microscopy and fluorescence spectroscopy of the membrane distribution of influenza hemagglutinin. *J. Cell Biol.* **2005**, *169*, 965–976. [[CrossRef](#)]

131. Scolari, S.; Engel, S.; Krebs, N.; Plazzo, A.P.; De Almeida, R.F.; Prieto, M.; Veit, M.; Herrmann, A. Lateral distribution of the transmembrane domain of influenza virus hemagglutinin revealed by time-resolved fluorescence imaging. *J. Biol. Chem.* **2009**, *284*, 15708–15716. [[CrossRef](#)]
132. Wilson, R.L.; Frisz, J.F.; Klitzing, H.A.; Zimmerberg, J.; Weber, P.K.; Kraft, M.L. Hemagglutinin clusters in the plasma membrane are not enriched with cholesterol and sphingolipids. *Biophys. J.* **2015**, *108*, 1652–1659. [[CrossRef](#)]
133. Chlanda, P.; Zimmerberg, J. Protein-lipid interactions critical to replication of the influenza A virus. *FEBS Lett.* **2016**, *590*, 1940–1954. [[CrossRef](#)] [[PubMed](#)]
134. Liu, Y.; Casey, L.; Pike, L.J. Compartmentalization of phosphatidylinositol 4,5-bisphosphate in low-density membrane domains in the absence of caveolin. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 684–690. [[CrossRef](#)] [[PubMed](#)]
135. Hope, H.R.; Pike, L.J. Phosphoinositides and phosphoinositide-utilizing enzymes in detergent-insoluble lipid domains. *Mol. Biol. Cell* **1996**, *7*, 843–851. [[CrossRef](#)] [[PubMed](#)]
136. Pike, L.J. Lipid rafts: Bringing order to chaos. *J. Lipid Res.* **2003**, *44*, 655–667. [[CrossRef](#)]
137. Raut, P.; Obeng, B.; Waters, H.; Zimmerberg, J.; Gosse, J.A.; Hess, S.T. Phosphatidylinositol 4,5-Bisphosphate Mediates the Co-Distribution of Influenza A Hemagglutinin and Matrix Protein M1 at the Plasma Membrane. *Viruses* **2022**, *14*, 2509. [[CrossRef](#)]
138. Curthoys, N.M.; Mlodzianowski, M.J.; Parent, M.; Butler, M.B.; Raut, P.; Wallace, J.; Lilieholm, J.; Mehmood, K.; Maginnis, M.S.; Waters, H.; et al. Influenza Hemagglutinin Modulates Phosphatidylinositol 4,5-Bisphosphate Membrane Clustering. *Biophys. J.* **2019**, *116*, 893–909. [[CrossRef](#)]
139. Tatulian, S.A.; Tamm, L.K. Secondary structure, orientation, oligomerization, and lipid interactions of the transmembrane domain of influenza hemagglutinin. *Biochemistry* **2000**, *39*, 496–507. [[CrossRef](#)]
140. Mineev, K.S.; Lyukmanova, E.N.; Krabben, L.; Serebryakova, M.V.; Shulepko, M.A.; Arseniev, A.S.; Kordyukova, L.V.; Veit, M. Structural investigation of influenza virus hemagglutinin membrane-anchoring peptide. *Protein Eng. Des. Sel.* **2013**, *26*, 547–552. [[CrossRef](#)]
141. Ngo, V.N.; Winski, D.P.; Aho, B.; Kamath, P.L.; King, B.L.; Waters, H.; Zimmerberg, J.; Sodt, A.; Hess, S.T. Conserved sequence features in intracellular domains of viral spike proteins. *Virology* **2024**, *599*, 110198. [[CrossRef](#)]
142. Gudheti, M.V.; Curthoys, N.M.; Gould, T.J.; Kim, D.; Gunewardene, M.S.; Gabor, K.A.; Gosse, J.A.; Kim, C.H.; Zimmerberg, J.; Hess, S.T. Actin mediates the nanoscale membrane organization of the clustered membrane protein influenza hemagglutinin. *Biophys. J.* **2013**, *104*, 2182–2192. [[CrossRef](#)]
143. Zhang, J.; Lamb, R.A. Characterization of the membrane association of the influenza virus matrix protein in living cells. *Virology* **1996**, *225*, 255–266. [[CrossRef](#)] [[PubMed](#)]
144. Peukes, J.; Xiong, X.; Erlendsson, S.; Qu, K.; Wan, W.; Calder, L.J.; Schraidt, O.; Kummer, S.; Freund, S.M.V.; Krausslich, H.G.; et al. The native structure of the assembled matrix protein 1 of influenza A virus. *Nature* **2020**, *587*, 495–498. [[CrossRef](#)] [[PubMed](#)]
145. Hofer, C.T.; Di Lella, S.; Dahmani, I.; Jungnick, N.; Bordag, N.; Bobone, S.; Huang, Q.; Keller, S.; Herrmann, A.; Chiantia, S. Structural determinants of the interaction between influenza A virus matrix protein M1 and lipid membranes. *Biochim. Biophys. Acta Biomembr.* **2019**, *1861*, 1123–1134. [[CrossRef](#)] [[PubMed](#)]
146. Kerviel, A.; Dash, S.; Moncorge, O.; Panthu, B.; Prchal, J.; Decimo, D.; Ohlmann, T.; Lina, B.; Favard, C.; Decroly, E.; et al. Involvement of an Arginine Triplet in M1 Matrix Protein Interaction with Membranes and in M1 Recruitment into Virus-Like Particles of the Influenza A(H1N1)pdm09 Virus. *PLoS ONE* **2016**, *11*, e0165421. [[CrossRef](#)]
147. Ruigrok, R.W.; Barge, A.; Durrer, P.; Brunner, J.; Ma, K.; Whittaker, G.R. Membrane interaction of influenza virus M1 protein. *Virology* **2000**, *267*, 289–298. [[CrossRef](#)]
148. Baudin, F.; Petit, I.; Weissenhorn, W.; Ruigrok, R.W. In vitro dissection of the membrane and RNP binding activities of influenza virus M1 protein. *Virology* **2001**, *281*, 102–108. [[CrossRef](#)]
149. Das, S.C.; Watanabe, S.; Hatta, M.; Noda, T.; Neumann, G.; Ozawa, M.; Kawaoka, Y. The highly conserved arginine residues at positions 76 through 78 of influenza A virus matrix protein M1 play an important role in viral replication by affecting the intracellular localization of M1. *J. Virol.* **2012**, *86*, 1522–1530. [[CrossRef](#)]
150. Thaa, B.; Herrmann, A.; Veit, M. The polybasic region is not essential for membrane binding of the matrix protein M1 of influenza virus. *Virology* **2009**, *383*, 150–155. [[CrossRef](#)]
151. Dahmani, I.; Ludwig, K.; Chiantia, S. Influenza A matrix protein M1 induces lipid membrane deformation via protein multimerization. *Biosci. Rep.* **2019**, *39*, BSR20191024. [[CrossRef](#)]
152. Shtykova, E.V.; Dadinova, L.A.; Fedorova, N.V.; Golanikov, A.E.; Bogacheva, E.N.; Ksenofontov, A.L.; Baratova, L.A.; Shilova, L.A.; Tashkin, V.Y.; Galimzyanov, T.R.; et al. Influenza virus Matrix Protein M1 preserves its conformation with pH, changing multimerization state at the priming stage due to electrostatics. *Sci. Rep.* **2017**, *7*, 16793. [[CrossRef](#)]
153. Hilsch, M.; Goldenbogen, B.; Sieben, C.; Hofer, C.T.; Rabe, J.P.; Klipp, E.; Herrmann, A.; Chiantia, S. Influenza A matrix protein M1 multimerizes upon binding to lipid membranes. *Biophys. J.* **2014**, *107*, 912–923. [[CrossRef](#)] [[PubMed](#)]
154. Kordyukova, L.V.; Konarev, P.V.; Fedorova, N.V.; Shtykova, E.V.; Ksenofontov, A.L.; Loshkarev, N.A.; Dadinova, L.A.; Timofeeva, T.A.; Abramchuk, S.S.; Moiseenko, A.V.; et al. The Cytoplasmic Tail of Influenza A Virus Hemagglutinin and Membrane Lipid Composition Change the Mode of M1 Protein Association with the Lipid Bilayer. *Membranes* **2021**, *11*, 772. [[CrossRef](#)] [[PubMed](#)]
155. Bobone, S.; Hilsch, M.; Storm, J.; Dunsing, V.; Herrmann, A.; Chiantia, S. Phosphatidylserine Lateral Organization Influences the Interaction of Influenza Virus Matrix Protein 1 with Lipid Membranes. *J. Virol.* **2017**, *91*, e00267-17. [[CrossRef](#)] [[PubMed](#)]

156. Loshkareva, A.S.; Popova, M.M.; Shilova, L.A.; Fedorova, N.V.; Timofeeva, T.A.; Galimzyanov, T.R.; Kuzmin, P.I.; Knyazev, D.G.; Batishchev, O.V. Influenza A Virus M1 Protein Non-Specifically Deforms Charged Lipid Membranes and Specifically Interacts with the Raft Boundary. *Membranes* **2023**, *13*, 76. [[CrossRef](#)]
157. Sha, B.; Luo, M. Structure of a bifunctional membrane-RNA binding protein, influenza virus matrix protein M1. *Nat. Struct. Biol.* **1997**, *4*, 239–244. [[CrossRef](#)]
158. Kakisaka, M.; Yamada, K.; Yamaji-Hasegawa, A.; Kobayashi, T.; Aida, Y. Intrinsically disordered region of influenza A NP regulates viral genome packaging via interactions with viral RNA and host PI(4,5)P2. *Virology* **2016**, *496*, 116–126. [[CrossRef](#)]
159. Gottlinger, H.G.; Sodroski, J.G.; Haseltine, W.A. Role of capsid precursor processing and myristoylation in morphogenesis and infectivity of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 5781–5785. [[CrossRef](#)]
160. Bryant, M.; Ratner, L. Myristoylation-dependent replication and assembly of human immunodeficiency virus 1. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 523–527. [[CrossRef](#)]
161. Zhou, W.; Parent, L.J.; Wills, J.W.; Resh, M.D. Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. *J. Virol.* **1994**, *68*, 2556–2569. [[CrossRef](#)]
162. Yuan, X.; Yu, X.; Lee, T.H.; Essex, M. Mutations in the N-terminal region of human immunodeficiency virus type 1 matrix protein block intracellular transport of the Gag precursor. *J. Virol.* **1993**, *67*, 6387–6394. [[CrossRef](#)]
163. Hermida-Matsumoto, L.; Resh, M.D. Human immunodeficiency virus type 1 protease triggers a myristoyl switch that modulates membrane binding of Pr55(gag) and p17MA. *J. Virol.* **1999**, *73*, 1902–1908. [[CrossRef](#)] [[PubMed](#)]
164. Paillart, J.C.; Gottlinger, H.G. Opposing effects of human immunodeficiency virus type 1 matrix mutations support a myristyl switch model of gag membrane targeting. *J. Virol.* **1999**, *73*, 2604–2612. [[CrossRef](#)] [[PubMed](#)]
165. Saad, J.S.; Loeliger, E.; Luncsford, P.; Liriano, M.; Tai, J.; Kim, A.; Miller, J.; Joshi, A.; Freed, E.O.; Summers, M.F. Point mutations in the HIV-1 matrix protein turn off the myristyl switch. *J. Mol. Biol.* **2007**, *366*, 574–585. [[CrossRef](#)] [[PubMed](#)]
166. Spearman, P.; Horton, R.; Ratner, L.; Kuli-Zade, I. Membrane binding of human immunodeficiency virus type 1 matrix protein in vivo supports a conformational myristyl switch mechanism. *J. Virol.* **1997**, *71*, 6582–6592. [[CrossRef](#)] [[PubMed](#)]
167. Ono, A.; Freed, E.O. Binding of human immunodeficiency virus type 1 Gag to membrane: Role of the matrix amino terminus. *J. Virol.* **1999**, *73*, 4136–4144. [[CrossRef](#)]
168. Tang, C.; Loeliger, E.; Luncsford, P.; Kinde, I.; Beckett, D.; Summers, M.F. Entropic switch regulates myristate exposure in the HIV-1 matrix protein. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 517–522. [[CrossRef](#)]
169. Freed, E.O.; Orenstein, J.M.; Buckler-White, A.J.; Martin, M.A. Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. *J. Virol.* **1994**, *68*, 5311–5320. [[CrossRef](#)]
170. Ono, A.; Orenstein, J.M.; Freed, E.O. Role of the Gag matrix domain in targeting human immunodeficiency virus type 1 assembly. *J. Virol.* **2000**, *74*, 2855–2866. [[CrossRef](#)]
171. Kleinpeter, A.B.; Freed, E.O. HIV-1 Maturation: Lessons Learned from Inhibitors. *Viruses* **2020**, *12*, 940. [[CrossRef](#)]
172. Thornhill, D.; Murakami, T.; Ono, A. Rendezvous at Plasma Membrane: Cellular Lipids and tRNA Set up Sites of HIV-1 Particle Assembly and Incorporation of Host Transmembrane Proteins. *Viruses* **2020**, *12*, 842. [[CrossRef](#)]
173. Ono, A.; Ablan, S.D.; Lockett, S.J.; Nagashima, K.; Freed, E.O. Phosphatidylinositol (4,5) bisphosphate regulates HIV-1 Gag targeting to the plasma membrane. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14889–14894. [[CrossRef](#)] [[PubMed](#)]
174. Chan, R.; Uchil, P.D.; Jin, J.; Shui, G.; Ott, D.E.; Mothes, W.; Wenk, M.R. Retroviruses human immunodeficiency virus and murine leukemia virus are enriched in phosphoinositides. *J. Virol.* **2008**, *82*, 11228–11238. [[CrossRef](#)] [[PubMed](#)]
175. Monde, K.; Chukkappalli, V.; Ono, A. Assembly and replication of HIV-1 in T cells with low levels of phosphatidylinositol-(4,5)-bisphosphate. *J. Virol.* **2011**, *85*, 3584–3595. [[CrossRef](#)]
176. Chan, J.; Dick, R.A.; Vogt, V.M. Rous sarcoma virus gag has no specific requirement for phosphatidylinositol-(4,5)-bisphosphate for plasma membrane association in vivo or for liposome interaction in vitro. *J. Virol.* **2011**, *85*, 10851–10860. [[CrossRef](#)]
177. Mucksch, F.; Laketa, V.; Muller, B.; Schultz, C.; Krausslich, H.G. Synchronized HIV assembly by tunable PIP(2) changes reveals PIP(2) requirement for stable Gag anchoring. *eLife* **2017**, *6*, e25287. [[CrossRef](#)]
178. Chukkappalli, V.; Hogue, I.B.; Boyko, V.; Hu, W.S.; Ono, A. Interaction between the human immunodeficiency virus type 1 Gag matrix domain and phosphatidylinositol-(4,5)-bisphosphate is essential for efficient gag membrane binding. *J. Virol.* **2008**, *82*, 2405–2417. [[CrossRef](#)]
179. Gerber, P.P.; Cabrini, M.; Jancic, C.; Paoletti, L.; Banchio, C.; von Bilderling, C.; Sigaut, L.; Pietrasanta, L.I.; Duette, G.; Freed, E.O.; et al. Rab27a controls HIV-1 assembly by regulating plasma membrane levels of phosphatidylinositol 4,5-bisphosphate. *J. Cell Biol.* **2015**, *209*, 435–452. [[CrossRef](#)]
180. Gonzales, B.; de Rocquigny, H.; Beziau, A.; Durand, S.; Burlaud-Gaillard, J.; Lefebvre, A.; Krull, S.; Emond, P.; Brand, D.; Piver, E. Type I Phosphatidylinositol-4-Phosphate 5-Kinases alpha and gamma Play a Key Role in Targeting HIV-1 Pr55(Gag) to the Plasma Membrane. *J. Virol.* **2020**, *94*, e00189-20. [[CrossRef](#)]
181. Thornhill, D.; Olety, B.; Ono, A. Relationships between MA-RNA Binding in Cells and Suppression of HIV-1 Gag Mislocalization to Intracellular Membranes. *J. Virol.* **2019**, *93*, e00756-19. [[CrossRef](#)]
182. Chukkappalli, V.; Oh, S.J.; Ono, A. Opposing mechanisms involving RNA and lipids regulate HIV-1 Gag membrane binding through the highly basic region of the matrix domain. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1600–1605. [[CrossRef](#)]

183. Mercredi, P.Y.; Bucca, N.; Loeliger, B.; Gaines, C.R.; Mehta, M.; Bhargava, P.; Tedbury, P.R.; Charlier, L.; Floquet, N.; Muriaux, D.; et al. Structural and Molecular Determinants of Membrane Binding by the HIV-1 Matrix Protein. *J. Mol. Biol.* **2016**, *428*, 1637–1655. [[CrossRef](#)] [[PubMed](#)]
184. Murphy, R.E.; Samal, A.B.; Vlach, J.; Mas, V.; Prevelige, P.E.; Saad, J.S. Structural and biophysical characterizations of HIV-1 matrix trimer binding to lipid nanodiscs shed light on virus assembly. *J. Biol. Chem.* **2019**, *294*, 18600–18612. [[CrossRef](#)] [[PubMed](#)]
185. Junkova, P.; Pleskot, R.; Prchal, J.; Sys, J.; Ruml, T. Differences and commonalities in plasma membrane recruitment of the two morphogenetically distinct retroviruses HIV-1 and MMTV. *J. Biol. Chem.* **2020**, *295*, 8819–8833. [[CrossRef](#)]
186. Barros, M.; Heinrich, F.; Datta, S.A.K.; Rein, A.; Karageorgos, I.; Nanda, H.; Lösche, M. Membrane Binding of HIV-1 Matrix Protein: Dependence on Bilayer Composition and Protein Lipidation. *J. Virol.* **2016**, *90*, 4544–4555. [[CrossRef](#)]
187. Keller, H.; Krausslich, H.G.; Schwille, P. Multimerizable HIV Gag derivative binds to the liquid-disordered phase in model membranes. *Cell. Microbiol.* **2013**, *15*, 237–247. [[CrossRef](#)]
188. Tran, R.J.; Lalonde, M.S.; Sly, K.L.; Conboy, J.C. Mechanistic Investigation of HIV-1 Gag Association with Lipid Membranes. *J. Phys. Chem. B* **2019**, *123*, 4673–4687. [[CrossRef](#)]
189. Carlson, L.A.; Hurley, J.H. In vitro reconstitution of the ordered assembly of the endosomal sorting complex required for transport at membrane-bound HIV-1 Gag clusters. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 16928–16933. [[CrossRef](#)]
190. Saad, J.S.; Miller, J.; Tai, J.; Kim, A.; Ghanam, R.H.; Summers, M.F. Structural basis for targeting HIV-1 Gag proteins to the plasma membrane for virus assembly. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11364–11369. [[CrossRef](#)]
191. Qu, K.; Ke, Z.; Zila, V.; Anders-Osswein, M.; Glass, B.; Mucksch, F.; Muller, R.; Schultz, C.; Muller, B.; Krausslich, H.G.; et al. Maturation of the matrix and viral membrane of HIV-1. *Science* **2021**, *373*, 700–704. [[CrossRef](#)]
192. Olety, B.; Veatch, S.L.; Ono, A. Phosphatidylinositol-(4,5)-Bisphosphate Acyl Chains Differentiate Membrane Binding of HIV-1 Gag from That of the Phospholipase Cdelta1 Pleckstrin Homology Domain. *J. Virol.* **2015**, *89*, 7861–7873. [[CrossRef](#)]
193. Lochrie, M.A.; Waugh, S.; Pratt, D.G., Jr.; Clever, J.; Parslow, T.G.; Polisky, B. In vitro selection of RNAs that bind to the human immunodeficiency virus type-1 gag polyprotein. *Nucleic Acids Res.* **1997**, *25*, 2902–2910. [[CrossRef](#)] [[PubMed](#)]
194. Purohit, P.; Dupont, S.; Stevenson, M.; Green, M.R. Sequence-specific interaction between HIV-1 matrix protein and viral genomic RNA revealed by in vitro genetic selection. *RNA* **2001**, *7*, 576–584. [[CrossRef](#)]
195. Cimarelli, A.; Luban, J. Translation elongation factor 1-alpha interacts specifically with the human immunodeficiency virus type 1 Gag polyprotein. *J. Virol.* **1999**, *73*, 5388–5401. [[CrossRef](#)]
196. Ramalingam, D.; Duclair, S.; Datta, S.A.; Ellington, A.; Rein, A.; Prasad, V.R. RNA aptamers directed to human immunodeficiency virus type 1 Gag polyprotein bind to the matrix and nucleocapsid domains and inhibit virus production. *J. Virol.* **2011**, *85*, 305–314. [[CrossRef](#)]
197. Jones, C.P.; Datta, S.A.; Rein, A.; Rouzina, I.; Musier-Forsyth, K. Matrix domain modulates HIV-1 Gag's nucleic acid chaperone activity via inositol phosphate binding. *J. Virol.* **2011**, *85*, 1594–1603. [[CrossRef](#)]
198. Hearps, A.C.; Wagstaff, K.M.; Piller, S.C.; Jans, D.A. The N-terminal basic domain of the HIV-1 matrix protein does not contain a conventional nuclear localization sequence but is required for DNA binding and protein self-association. *Biochemistry* **2008**, *47*, 2199–2210. [[CrossRef](#)]
199. Alfadhli, A.; Still, A.; Barklis, E. Analysis of human immunodeficiency virus type 1 matrix binding to membranes and nucleic acids. *J. Virol.* **2009**, *83*, 12196–12203. [[CrossRef](#)]
200. Socas, L.B.P.; Ambroggio, E.E. HIV-1 Gag specificity for PIP(2) is regulated by macromolecular electric properties of both protein and membrane local environments. *Biochim. Biophys. Acta Biomembr.* **2023**, *1865*, 184157. [[CrossRef](#)]
201. Sumner, C.; Kotani, O.; Liu, S.; Musier-Forsyth, K.; Sato, H.; Ono, A. Molecular Determinants in tRNA D-arm Required for Inhibition of HIV-1 Gag Membrane Binding. *J. Mol. Biol.* **2022**, *434*, 167390. [[CrossRef](#)]
202. Todd, G.C.; Duchon, A.; Inlora, J.; Olson, E.D.; Musier-Forsyth, K.; Ono, A. Inhibition of HIV-1 Gag-membrane interactions by specific RNAs. *RNA* **2017**, *23*, 395–405. [[CrossRef](#)]
203. Inlora, J.; Collins, D.R.; Trubin, M.E.; Chung, J.Y.; Ono, A. Membrane binding and subcellular localization of retroviral Gag proteins are differentially regulated by MA interactions with phosphatidylinositol-(4,5)-bisphosphate and RNA. *mBio* **2014**, *5*, e02202. [[CrossRef](#)]
204. Chukkapalli, V.; Inlora, J.; Todd, G.C.; Ono, A. Evidence in support of RNA-mediated inhibition of phosphatidylserine-dependent HIV-1 Gag membrane binding in cells. *J. Virol.* **2013**, *87*, 7155–7159. [[CrossRef](#)] [[PubMed](#)]
205. Kutluay, S.B.; Zang, T.; Blanco-Melo, D.; Powell, C.; Jannain, D.; Errando, M.; Bieniasz, P.D. Global changes in the RNA binding specificity of HIV-1 gag regulate virion genesis. *Cell* **2014**, *159*, 1096–1109. [[CrossRef](#)] [[PubMed](#)]
206. Inlora, J.; Chukkapalli, V.; Derse, D.; Ono, A. Gag localization and virus-like particle release mediated by the matrix domain of human T-lymphotropic virus type 1 Gag are less dependent on phosphatidylinositol-(4,5)-bisphosphate than those mediated by the matrix domain of HIV-1 Gag. *J. Virol.* **2011**, *85*, 3802–3810. [[CrossRef](#)]
207. Dick, R.A.; Kamynina, E.; Vogt, V.M. Effect of multimerization on membrane association of Rous sarcoma virus and HIV-1 matrix domain proteins. *J. Virol.* **2013**, *87*, 13598–13608. [[CrossRef](#)] [[PubMed](#)]
208. Alfadhli, A.; McNett, H.; Tsagli, S.; Bachinger, H.P.; Peyton, D.H.; Barklis, E. HIV-1 matrix protein binding to RNA. *J. Mol. Biol.* **2011**, *410*, 653–666. [[CrossRef](#)]

209. Gaines, C.R.; Tkacik, E.; Rivera-Oven, A.; Somani, P.; Achimovich, A.; Alabi, T.; Zhu, A.; Getachew, N.; Yang, A.L.; McDonough, M.; et al. HIV-1 Matrix Protein Interactions with tRNA: Implications for Membrane Targeting. *J. Mol. Biol.* **2018**, *430*, 2113–2127. [[CrossRef](#)]
210. Bou-Nader, C.; Muecksch, F.; Brown, J.B.; Gordon, J.M.; York, A.; Peng, C.; Ghirlando, R.; Summers, M.F.; Bieniasz, P.D.; Zhang, J. HIV-1 matrix-tRNA complex structure reveals basis for host control of Gag localization. *Cell Host Microbe* **2021**, *29*, 1421–1436.e7. [[CrossRef](#)]
211. Charlier, L.; Louet, M.; Chaloin, L.; Fuchs, P.; Martinez, J.; Muriaux, D.; Favard, C.; Floquet, N. Coarse-grained simulations of the HIV-1 matrix protein anchoring: Revisiting its assembly on membrane domains. *Biophys. J.* **2014**, *106*, 577–585. [[CrossRef](#)]
212. Banerjee, P.; Qu, K.; Briggs, J.A.G.; Voth, G.A. Molecular dynamics simulations of HIV-1 matrix-membrane interactions at different stages of viral maturation. *Biophys. J.* **2024**, *123*, 389–406. [[CrossRef](#)]
213. Favard, C.; Chojnacki, J.; Merida, P.; Yandrapalli, N.; Mak, J.; Eggeling, C.; Muriaux, D. HIV-1 Gag specifically restricts PI(4,5)P2 and cholesterol mobility in living cells creating a nanodomain platform for virus assembly. *Sci. Adv.* **2019**, *5*, eaaw8651. [[CrossRef](#)] [[PubMed](#)]
214. Mucksch, F.; Citir, M.; Luchtenborg, C.; Glass, B.; Traynor-Kaplan, A.; Schultz, C.; Brugger, B.; Krausslich, H.G. Quantification of phosphoinositides reveals strong enrichment of PIP(2) in HIV-1 compared to producer cell membranes. *Sci. Rep.* **2019**, *9*, 17661. [[CrossRef](#)] [[PubMed](#)]
215. Wen, Y.; Feigenson, G.W.; Vogt, V.M.; Dick, R.A. Mechanisms of PI(4,5)P2 Enrichment in HIV-1 Viral Membranes. *J. Mol. Biol.* **2020**, *432*, 5343–5364. [[CrossRef](#)]
216. Yandrapalli, N.; Lubart, Q.; Tanwar, H.S.; Picart, C.; Mak, J.; Muriaux, D.; Favard, C. Self assembly of HIV-1 Gag protein on lipid membranes generates PI(4,5)P(2)/Cholesterol nanoclusters. *Sci. Rep.* **2016**, *6*, 39332. [[CrossRef](#)]
217. Grover, J.R.; Veatch, S.L.; Ono, A. Basic motifs target PSGL-1, CD43, and CD44 to plasma membrane sites where HIV-1 assembles. *J. Virol.* **2015**, *89*, 454–467. [[CrossRef](#)]
218. Murakami, T.; Carmona, N.; Ono, A. Virion-incorporated PSGL-1 and CD43 inhibit both cell-free infection and transinfection of HIV-1 by preventing virus-cell binding. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 8055–8063. [[CrossRef](#)]
219. Murakami, T.; Kim, J.; Li, Y.; Green, G.E.; Shikanov, A.; Ono, A. Secondary lymphoid organ fibroblastic reticular cells mediate trans-infection of HIV-1 via CD44-hyaluronan interactions. *Nat. Commun.* **2018**, *9*, 2436. [[CrossRef](#)]
220. Murakami, T.; Ono, A. Roles of Virion-Incorporated CD162 (PSGL-1), CD43, and CD44 in HIV-1 Infection of T Cells. *Viruses* **2021**, *13*, 1935. [[CrossRef](#)]
221. Fu, Y.; He, S.; Waheed, A.A.; Dabbagh, D.; Zhou, Z.; Trinite, B.; Wang, Z.; Yu, J.; Wang, D.; Li, F.; et al. PSGL-1 restricts HIV-1 infectivity by blocking virus particle attachment to target cells. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 9537–9545. [[CrossRef](#)]
222. Liu, Y.; Song, Y.; Zhang, S.; Diao, M.; Huang, S.; Li, S.; Tan, X. PSGL-1 inhibits HIV-1 infection by restricting actin dynamics and sequestering HIV envelope proteins. *Cell Discov.* **2020**, *6*, 53. [[CrossRef](#)]
223. Liu, Y.; Fu, Y.; Wang, Q.; Li, M.; Zhou, Z.; Dabbagh, D.; Fu, C.; Zhang, H.; Li, S.; Zhang, T.; et al. Proteomic profiling of HIV-1 infection of human CD4(+) T cells identifies PSGL-1 as an HIV restriction factor. *Nat. Microbiol.* **2019**, *4*, 813–825. [[CrossRef](#)]
224. Cardoso, R.d.S.; Murakami, T.; Jacobovitz, B.; Veatch, S.L.; Ono, A. PI(4,5)P2 promotes the incorporation of CD43, PSGL-1 and CD44 into nascent HIV-1 particles. *bioRxiv* 2024. [[CrossRef](#)]
225. Dalton, A.K.; Ako-Adjei, D.; Murray, P.S.; Murray, D.; Vogt, V.M. Electrostatic interactions drive membrane association of the human immunodeficiency virus type 1 Gag MA domain. *J. Virol.* **2007**, *81*, 6434–6445. [[CrossRef](#)]
226. Vlach, J.; Saad, J.S. Trio engagement via plasma membrane phospholipids and the myristoyl moiety governs HIV-1 matrix binding to bilayers. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3525–3530. [[CrossRef](#)]
227. Monje-Galvan, V.; Voth, G.A. Binding mechanism of the matrix domain of HIV-1 gag on lipid membranes. *eLife* **2020**, *9*, e58621. [[CrossRef](#)]
228. Sumner, C.; Ono, A. The “basics” of HIV-1 assembly. *PLoS Pathog.* **2024**, *20*, e1011937. [[CrossRef](#)]
229. Chua, B.A.; Ngo, J.A.; Situ, K.; Morizono, K. Roles of phosphatidylserine exposed on the viral envelope and cell membrane in HIV-1 replication. *Cell Commun. Signal.* **2019**, *17*, 132. [[CrossRef](#)]
230. Moller-Tank, S.; Maury, W. Phosphatidylserine receptors: Enhancers of enveloped virus entry and infection. *Virology* **2014**, *468–470*, 565–580. [[CrossRef](#)]
231. Callahan, M.K.; Popernack, P.M.; Tsutsui, S.; Truong, L.; Schlegel, R.A.; Henderson, A.J. Phosphatidylserine on HIV envelope is a cofactor for infection of monocytic cells. *J. Immunol.* **2003**, *170*, 4840–4845. [[CrossRef](#)]
232. Fadok, V.A.; Voelker, D.R.; Campbell, P.A.; Cohen, J.J.; Bratton, D.L.; Henson, P.M. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* **1992**, *148*, 2207–2216. [[CrossRef](#)]
233. Segawa, K.; Nagata, S. An Apoptotic ‘Eat Me’ Signal: Phosphatidylserine Exposure. *Trends Cell Biol.* **2015**, *25*, 639–650. [[CrossRef](#)]
234. Mercer, J.; Helenius, A. Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science* **2008**, *320*, 531–535. [[CrossRef](#)]
235. Mercer, J.; Knebel, S.; Schmidt, F.I.; Crouse, J.; Burkard, C.; Helenius, A. Vaccinia virus strains use distinct forms of macropinocytosis for host-cell entry. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 9346–9351. [[CrossRef](#)]
236. Li, M.; Ablan, S.D.; Miao, C.; Zheng, Y.M.; Fuller, M.S.; Rennert, P.D.; Maury, W.; Johnson, M.C.; Freed, E.O.; Liu, S.L. TIM-family proteins inhibit HIV-1 release. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E3699–E3707. [[CrossRef](#)]

237. Rosa, A.; Chande, A.; Ziglio, S.; De Sanctis, V.; Bertorelli, R.; Goh, S.L.; McCauley, S.M.; Nowosielska, A.; Antonarakis, S.E.; Luban, J.; et al. HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. *Nature* **2015**, *526*, 212–217. [[CrossRef](#)]
238. Usami, Y.; Wu, Y.; Gottlinger, H.G. SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. *Nature* **2015**, *526*, 218–223. [[CrossRef](#)]
239. Sid Ahmed, S.; Bajak, K.; Fackler, O.T. Beyond Impairment of Virion Infectivity: New Activities of the Anti-HIV Host Cell Factor SERINC5. *Viruses* **2024**, *16*, 284. [[CrossRef](#)]
240. Leonhardt, S.A.; Purdy, M.D.; Grover, J.R.; Yang, Z.; Poulos, S.; McIntire, W.E.; Tatham, E.A.; Erramilli, S.K.; Nosol, K.; Lai, K.K.; et al. Antiviral HIV-1 SERINC restriction factors disrupt virus membrane asymmetry. *Nat. Commun.* **2023**, *14*, 4368. [[CrossRef](#)]
241. Raghunath, G.; Abbott, E.H.; Marin, M.; Wu, H.; Reyes Ballista, J.M.; Brindley, M.A.; Melikyan, G.B. Disruption of Transmembrane Phosphatidylserine Asymmetry by HIV-1 Incorporated SERINC5 Is Not Responsible for Virus Restriction. *Biomolecules* **2024**, *14*, 570. [[CrossRef](#)]
242. Harrison, M.S.; Sakaguchi, T.; Schmitt, A.P. Paramyxovirus assembly and budding: Building particles that transmit infections. *Int. J. Biochem. Cell Biol.* **2010**, *42*, 1416–1429. [[CrossRef](#)]
243. Swain, J.; Bierre, M.; Veyrie, L.; Richard, C.A.; Eleouet, J.F.; Muriaux, D.; Bajorek, M. Selective targeting and clustering of phosphatidylserine lipids by RSV M protein is critical for virus particle production. *J. Biol. Chem.* **2023**, *299*, 105323. [[CrossRef](#)]
244. Wang, J.; Gambhir, A.; Hangyas-Mihalyne, G.; Murray, D.; Golebiewska, U.; McLaughlin, S. Lateral sequestration of phosphatidylinositol 4,5-bisphosphate by the basic effector domain of myristoylated alanine-rich C kinase substrate is due to nonspecific electrostatic interactions. *J. Biol. Chem.* **2002**, *277*, 34401–34412. [[CrossRef](#)]
245. Murphy, R.E.; Samal, A.B.; Vlach, J.; Saad, J.S. Solution Structure and Membrane Interaction of the Cytoplasmic Tail of HIV-1 gp41 Protein. *Structure* **2017**, *25*, 1708–1718.e05. [[CrossRef](#)]
246. Harris, A.; Cardone, G.; Winkler, D.C.; Heymann, J.B.; Brecher, M.; White, J.M.; Steven, A.C. Influenza virus pleiomorphy characterized by cryoelectron tomography. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 19123–19127. [[CrossRef](#)]
247. Calder, L.J.; Wasilewski, S.; Berriman, J.A.; Rosenthal, P.B. Structural organization of a filamentous influenza A virus. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10685–10690. [[CrossRef](#)]
248. Inamdar, K.; Tsai, F.C.; Dibsby, R.; de Poret, A.; Manzi, J.; Merida, P.; Muller, R.; Lappalainen, P.; Roingard, P.; Mak, J.; et al. Full assembly of HIV-1 particles requires assistance of the membrane curvature factor IRSp53. *eLife* **2021**, *10*, e67321.
249. Kumakura, M.; Kawaguchi, A.; Nagata, K. Actin-myosin network is required for proper assembly of influenza virus particles. *Virology* **2015**, *476*, 141–150. [[CrossRef](#)]
250. Bedi, S.; Ono, A. Friend or Foe: The Role of the Cytoskeleton in Influenza A Virus Assembly. *Viruses* **2019**, *11*, 46. [[CrossRef](#)]
251. Morizono, K.; Chen, I.S. Role of phosphatidylserine receptors in enveloped virus infection. *J. Virol.* **2014**, *88*, 4275–4290.
252. Amara, A.; Mercer, J. Viral apoptotic mimicry. *Nat. Rev. Microbiol.* **2015**, *13*, 461–469.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.