# Artificial Lipid Membranes for Viral Assembly Research

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Contributor: Cyril Favard , Erwan Brémaud , Delphine Muriaux

The cell plasma membrane is mainly composed of phospholipids, cholesterol and embedded proteins, presenting a complex interface with the cell environment. Enveloped viruses are also surrounded by a lipidic membrane derived from the host-cell membrane and acquired during the assembly at and the budding from the host cell plasma membrane. In this perspective, model membranes, composed of selected lipid mixtures mimicking plasma membrane chemical and physical properties, are tools of choice to decipher the first steps of enveloped viruses assembly. Hereafter are detailled some of the existing artificial lipid membranes and their contribution in deciphering the assembly process of 3 well known envelopped virus, the human immunodeficiency virus 1 (HIV-1), the Influenza virus (IfV) and the Ebola virus (EboV).

viral assembly | biomimetic membranes | membrane proteins | membrane dynamics | biophysical techniques

## 1. Simplified Overview of Viral Assembly

Viral assembly, which is mainly driven by the self-assembly of viral structural proteins, precedes new particle release from the host cell. In the case of HIV-1, for example, 5 min are required for the viral particle to assemble, whereas budding and particle release occur, on average, 15 min later, independently of the cell type [1][2]. Viral assembly can be seen as a protein polymerization process involving three main steps, namely initiation, elongation and termination. In the case of enveloped virus assembly, initiation is always difficult to define. Here, the researchers define initiation as the nucleation step, i.e., the generation of a nucleus containing a small number of viral and host components, as follows: in the case of HIV-1, structural group-specific antigen (Gag) proteins, the viral RNA genome and host-cell membrane lipids; and, in the case of Influenza, the viral M1 and M2 proteins and the host-cell plasma membrane phosphophatidylserine. Initiation/nucleation requires an energetic barrier to be overcome [3]. From this perspective, the membrane can act as a dimensional catalyzer, which increases the probability of viral protein/viral protein and/or viral protein/genome complex encounters, as well as favoring assembly through entropic effects. In the very first step in membrane assembly, viral proteins are recruited at the membrane, generally via the interaction of the proteins with charged phospholipids.

## 2. Langmuir Monolayers: A Fine Tuning System to Monitor Membrane-Viral Proteins Interactions

A Langmuir monolayer is composed of a single lipid layer at the interface between an aqueous environment and the air. However, unlike cellular PMs, it is very easy to tune its physical parameters, which makes this model system very useful to study protein–lipid interactions that require controlled membrane physical properties (i.e., the lipids' packing density within the monolayer, or the surface pressure applied to the layer).

The assembly of HIV-1 is mainly driven by viral Gag protein oligomerization. However, accessory proteins, such as the viral negative regulatory factor (Nef) protein, could play a secondary role during HIV-1 particle formation, although this role is still debated. Nef was described as promoting Gag membrane localization [4]. Pirrone *et al.* studied the conformation dependence of myristoylated Nef (myrNef) with lipid and packing density using hydrogen exchange mass spectrometry, or HX MS (see Lexicon for definition) on Langmuir monolayers [5]. They showed that myrNef undergoes a conformational change when the lipid density decreases, turning from a compact conformation adjacent to the membrane into a form in which the N-terminal arm is inserted into the membrane, causing Nef core displacement away from the membrane. This research suggested that, depending on its structure and on the membrane properties, Nef could perform different functions.

It is firmly established that VP40, the major Ebola Matrix protein, regulates virus assembly and egress at the inner leaflet of the host-cell plasma membrane by recognizing PS [6]. To identify the VP40 amino acids involved in membrane recognition, Adu-Gyamfi *et al.* generated several VP40 protein mutants and monitored their capacity to penetrate Langmuir monolayers by measuring changes in the monolayers' lateral pressure. These findings exhibited the involvement of a particular region in the C-terminus domain of VP40 in PM localization, but also in VP40 self-assembly and particle egress [7]. As such measures require fine pressure control over the lipids, Langmuir monolayers are the most appropriate model system to use.

## 3. Using Bicelles to Elucidate the Molecular Structures of Viral Proteins on Membranes

Bicelles represent a relatively small, minimal system of lipid bilayers, comprising flat disks of lipids with both sides surrounded by the same aqueous environment. They are widely used in structural/atomistic analyses of membrane binding proteins. On the other hand, three main methods are currently used to study protein structure (see **Figure 1**A): X-ray crystallography, cryo-electron microscopy and nuclear magnetic resonance (NMR). NMR is the only method that enables the studies at the atomistic level to be conducted in the solid state as well as in solution. NMR makes it possible to investigate protein dynamics, as well as structure, which gives access to the more intimate biological mechanisms of proteins. To gain an insight into the structure of the MA domain of HIV-1 Gag in interaction with the membrane, NMR experiments were initially performed in solution with di-C4 and di-C8 $PI(4,5)P_2$ lipids complexed to MA [8]. From these experiments, a structure was proposed, exhibiting a strong interaction of the highly basic region (HBR) of MA with the polar head of the $PI(4,5)P_2$, a switch of the myristoyl group of the MA from an hydrophobic pocket of the protein towards the exterior, supposedly to the PM and, at the same time, a swap of the 2′ acyl chain of the diC8-$PI(4,5)P_2$ into another hydrophobic pocket of the MA. However, since the NMR was performed in solution, these structural rearrangements could be questioned [9]. Subsequently, Vlach and Saad complemented their structural characterization by using diC8-$PI(4,5)P_2$ in addition to different diC6

acylated lipids (PhosphatidylCholine (PC), PhosphatidylEthanoamine (PE) and PhosphatidylSerine(PS)) [10]. They observed that not only did the 2′ acyl chain of the PI(4,5)$P_2$ flip into an MA hydrophobic pocket but that, moreover, in the case of PC, PS or PE, their 2′ acyl chains flipped into distinct MA hydrophobic pockets. More recently, thanks to the use of bicelles, Mercredi *et al.* performed NMR and revisited these previously established MA structures on membranes [11]. While they observed a strong interaction of the MA HBR with the polar head of the PI(4,5)$P_2$ and, to a lesser extent, an interaction with the PS head at the MA position identified by [10], they did not observe any flipping of the acyl chains of these lipids into any of the two hydrophobic pockets. This was previously suggested by a coarse-grained molecular dynamic model of HIV-1 MA on PI(4,5)$P_2$/PS membranes [12]. The added value of bicelles for NMR structure elucidation is clearly shown by this example, in which, when isolated, the phopholipid does not interact with the MA in the same way as when it is inserted into its natural environment, i.e., an amphiphilic membrane.

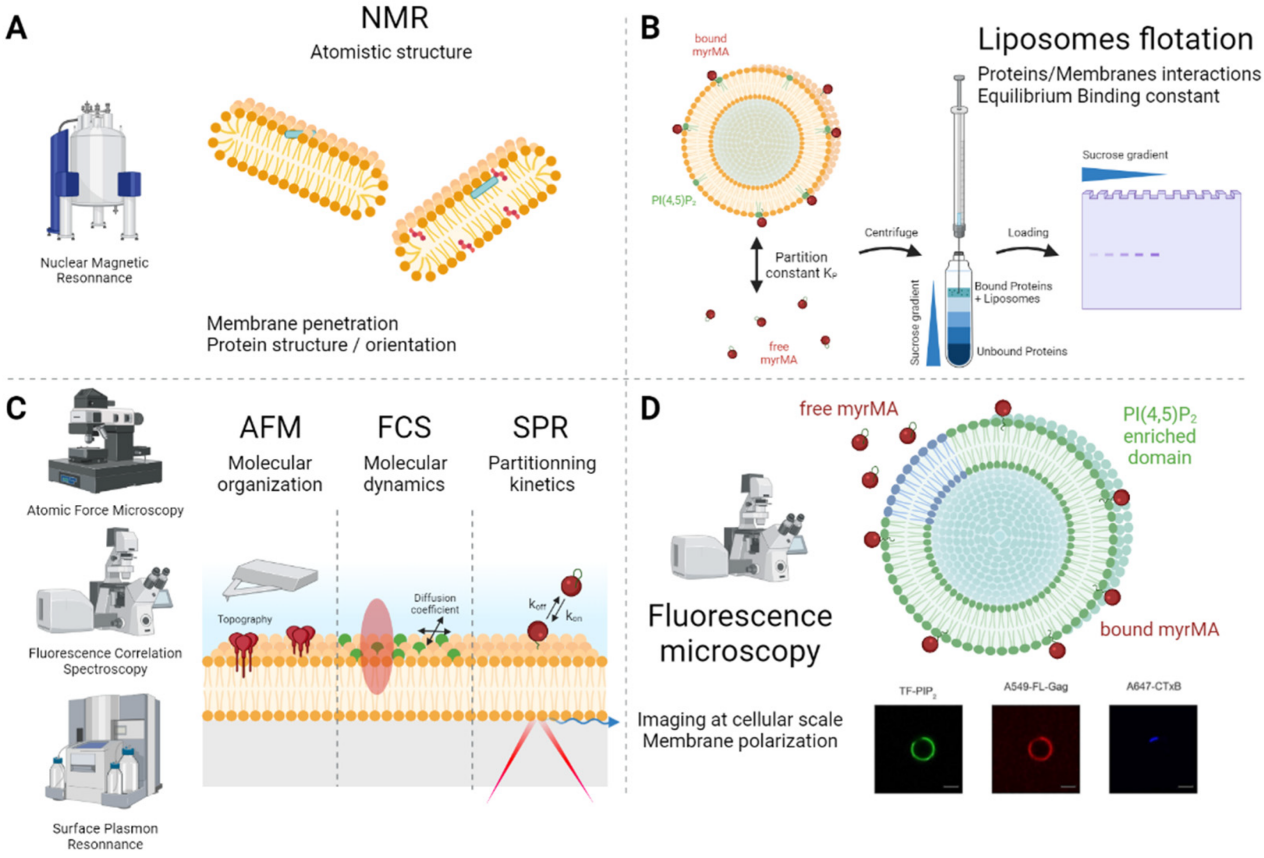


**Figure 1. Methods and techniques for viral assembly study in several membrane model systems.** (**A**) Bicelles are widely used models for protein structural analysis by NMR. (**B**) Liposome flotation unveils partition constant of a protein at a membrane's surface using LUVs or SUVs. (**C**) Using the SLB planar system, AFM gives topographic information at a molecular level, FCS enables study of molecular dynamics and SPR is a way of studying binding properties. (**D**) GUVs are close in size and shape to cells, and one can, for instance, image protein–-lipid co-localizations at a cellular scale.

Wang and Huong also used bicelles to investigate the membrane curvature induction by IAV M2 protein (a transmembrane viral channel that is important for virus entry and particle budding) using OMAS-NMR [13] (Off-axis Magic Angle Spinning NMR). They showed that the M2 amphipathic helix (M2 AH), along with its transmembrane domain (M2 TM), induced strong membrane curvature in the bicelles, which in turn favored and stabilized the localization of the M2 in these strongly curved domains. These findings highlight the functional role of M2 during IAV assembly and is involvement in membrane deformation to promote IAV particle budding (reviewed in [14]).

## 4. Unilamellar Vesicles

As they are easy to make, and their composition is easy to tune, large unilamellar vesicles (LUVs) have been widely used in the field of viral assembly.

Using LUVs, many different mechanisms of the HIV-1 Gag interaction with membranes were determined (**Figure 1**B). The residues of the matrix were identified as specific to the PI(4,5)$P_2$ interactions [15][16]. Preferential lipid compositions were tested in order to monitor the effect of the different lipids [17][18][19] and the main role of PI(4,5)$P_2$ was confirmed [20]. The roles of myristoylation and the electrostatic nature of the interaction were also identified and quantified using LUVs [16][21][22]. The binding equilibrium of the MA domain of the Gag between the membrane lipids and the RNA was also investigated thanks to LUVs. For example, Chukkapalli et al. highlighted the importance of five residues found to bind RNA, which also restricts MA binding to membranes lacking PI(4,5)$P_2$, supporting the role of RNA in masking the non-specific binding of Gag to membranes [23] and the regulation of membrane binding by t-RNA [24][25].

LUVs also help to understand the lipid organization/composition in the membrane that rules the assembly. For example, self-assembling matrix lipid-phase partitioning and lipid phase separation occurring during Gag self-assembly were investigated with LUVs [18][26]. Recently, Urbančič *et al.* [27] determined that lipid composition but not membrane curvature influenced HIV envelope-like lipid membrane fluidity by measuring the lipid diffusion through line-scanning stimulated emission depletion-fluorescence correlation spectroscopy (STED-FCS).

LUVs have heavily contributed to the understanding of HIV-1 Gag membrane interactions initiating the generation of new viruses, but they are also key tools to decipher IAV matrix protein/lipid membrane interactions.

Since the pioneering work of Gregoriades and Oxford [28][29], PS has been identified as the main target for the membrane association of IAV M1 [30][31][32]. Recently, the effect of lipid composition on the initiation of M1 self-assembly has been studied using SUVs (small unilamellar vesicles) and proteoliposomes, showing the role of lipid ordering in the association of M1 to PS in these liposomes [33]. Liposomes were also used to monitor the interactions of virus proteins such as HA [29] and NA [34] with lipids and lipid redistribution; however, this was essentially considered during membrane fusion [35], which is the entry step (early phase of replication) of the virus, not the assembly step. A significant effort has been directed at the understanding of the M2 protein's interaction with lipids in the case of IAV. This because M2 is a transmembrane protein and is described as playing a major role in the budding of the Influenza virus by inducing membrane scission [36]. Virus assembly and budding generate

changes in the local curvatures of membranes. At the end of assembly, a neck appears at the bud with a strong and unstable local curvature. In the case of IAV, M2, thanks to its amphipatic helix (AH), has been proposed to both sense and stabilize this local curvature [13]. This localization has been confirmed both by using liposomes of different curvatures [37] and by using liposomes containing different cholesterol concentrations, showing that cholesterol induces the reorientation of this amphipatic helix [38].

Ebola virus assembly is poorly studied with membrane model systems. However, 20 years ago, VP40 structure and hexamerization were studied using liposomes [39][40]. The C-terminal truncation of the VP40 protein was shown to be responsible for its spontaneous hexamerization, suggesting its main role in the initiation step of EBOV assembly. Scianimanico et al. observed, using the membrane flotation technique, that VP40 membrane association triggered hexamerization [40]. Using combination of surface plasmon resonance (SPR) and membrane flotation assays, the membrane binding of VP40 was shown to be essentially mediated by its interaction with PS [7] and $PI(4,5)P_2$ [41]; this was also confirmed in living cells in the same studies.

## 5. Supported and Tethered Bilayers: Planar Membranes

SLBs are planar lipid membranes lying on the surfaces of glass coverslips. One major advantage is that they offer a widely accessible environment in which to study protein binding, diffusion and the lipid-induced reorganization occurring simultaneously. A second advantage is the possibility of using a wide object only a few nm in height, allowing its direct observation at the molecular level by using techniques such as atomic force microscopy (AFM) (see **Figure 1**C). For these reasons, SLBs were used to study viral protein assembly using AFM [42].

Using SLBs with a lipid composition mimicking the inner leaflet of the cell plasma membrane, as well as measuring the self-quenching of fluorescent lipids, Yandrapalli et al. showed that, upon self-assembly, HIV-1 Gag generated $PI(4,5)P_2$/Cholesterol clusters [18]. This finding questioned the model suggesting that either HIV-1 Gag targeted the pre-enriched lipid domains of the membrane to self-assemble or generated its own lipid bed. Interestingly, in a subsequent study, this $PI(4,5)P_2$/Cholesterol nano-clustering was also shown to occur during the assembly of new HIV-1 viruses at the host plasma membrane of HIV-1-infected T cells [43], revealing that HIV-1 Gag self-assembly is the driving force in $PI(4,5)P_2$/Cholesterol-enriched lipid nanodomain generation.

It is commonly accepted that Gag-Gag interactions are sufficient to produce the energy required to initiate HIV-1 assembly. However, using atomic force microscopy (AFM) on SLBs, Miles et al. observed that interactions between HIV-1 envelope glycoproteins Gp41 and Gp120 could also partially drive viral assembly [44]. The SLBs were processed by vesicle fusion and Gp41 containing vesicles were fused to the SLBs. The Gp120 proteins were then consecutively injected and formed wire-shaped structures at the bilayer surface. The described interactions between these Gp proteins, although they were weaker and formed abnormally shaped assembling particles, could, according to the authors, be considered as a driving force in viral assembly.

S. Chiantia's group intensively used SLBs to explore IAV assembly by monitoring M1-M1 interactions with quantitative optical microscopy. Using RICS (Raster scan Imaging Correlation Spectroscopy) and FCS

(Fluorescence Correlation Spectroscopy), in addition to AFM (**Figure 1**C), Hilsch *et al.* described M1 self-assembly enhancement in PS-containing membranes [45]. They proposed that M1 self-assembly was sufficient to initiate the formation of new viral particles, even in the absence of other viral proteins.

These results were confirmed subsequently and the interplay between PS and M1, in which M1 interacts mostly with PS-enriched domains within lipid bilayers and stabilizes these PS domains during M1 self-assembly, was described in detail [46].

Finally, thanks to RICS, SPR and circular dichroism spectroscopy, a subsequent investigation of the precise mechanism of the interaction between IAV M1 protein and the PS-enriched bilayer was published by Höfer *et al.* in 2019 [47]. A specific conformational change was found to occur upon the M1's binding to the negatively charged PS. From the structures in the protein data bank (PDB) and simulations, N-terminal domains of M1 was shown to be involved in membrane binding, stabilizing C-terminal domain to favor self-assembly.

SLBs are standard models for lipid diffusion measurements. However, a two-to-threefold decrease in lipid diffusion coefficients is usually measured between SLBs and liposomes or GUVs. Indeed, Van der Waals interactions between the glass substrate and the lipids monolayer in contact with it led to an overall decrease in diffusion [48]. This decrease in lipid mobility in one monolayer could impede the lateral organization of lipids and therefore play an artificial role in viral protein membrane binding. To remove these Van der Waals interactions, a sparsely tethered bilayer lipid membrane (stBLM) system was used by Barros et al. to quantify, using SPR, HIV-1 Gag MA binding to the membrane. They showed that MA was attracted to the membrane by charged lipids, while the MA myristate exposure increased the membrane affinity 100-fold. They also highlighted that cholesterol facilitated myristate insertion and $PI(4,5)P_2$ binding without interacting with the MA. Interestingly, the concomitant role of cholesterol and $PI(4,5)P_2$ was also observed in [18] using classical SLBs, suggesting again that the lipid organization for correct binding and virus formation is mainly driven by Gag-Gag self-assembly and not by pre-existing lipid domains.

## 6. Giant Unilamellar Vesicles: The Closest Model to Cell Plasma Membranes

Amongst all the models used to mimic cellular membranes, GUVs have the closest properties to the cell plasma membrane. They are spherical lipid bilayers with a diameter range equivalent to that of a eukaryotic cell (~10 µm) enclosing and surrounded by an aqueous medium. Their similarities with eukaryotic cells make it possible to study, in GUVs, several molecular properties of membrane lipids and their interactions with viral proteins, such as lipid ordering, lipid–protein interaction localization, and curvature or tubular structure induction.

GUVs were used models to directly visualize HIV-1 Gag proteins' binding properties and localization in membranes with complex lipid compositions. When preparing GUVs with two separate phases, i.e., liquid-ordered and liquid-disordered, it was possible to show that the Gag, as well as a the multimerizing MA domain of the Gag, mainly partitioned into liquid-disordered phase where $PI(4,5)P_2$ was present and that this partitioning did not change upon Gag self-assembly [18][26] (**Figure 1**D). These results were confirmed recently using the same approach [49].

Another interesting point regarding GUVs is that their wide, flat lipid surfaces make it possible to study not only protein assembly initiation but also aspects of its termination, such as viral budding. Gui *et al.* developed a protocol using a GUV model without cellular proteins that makes it possible to infer that Gag proteins' self-aggregation alone leads to vesicle formation budding from the GUV membrane (an indirect observation based on GUV size-reduction measurements) [50]. However, in the late steps of retroviral assembly, the cellular endosomal sorting complex (ESCRT) machinery is recruited by Gag-p6 domain interacting with tumor susceptibility gene 101 (Tsg101) at HIV-1 lattices in order to facilitate virus budding [51]. Subsequently, using GUVs, Carlson and Hurley reconstituted an in vitro minimal system to monitor the dynamics of ESCRT machinery recruitment at HIV-1 Gag budding sites [52].

GUVs have also been extensively used to decipher Influenza virus assembly. Dahmani *et al.* observed, using confocal microscopy, that M1 binding to PS loaded GUVs induced the local deformation of the lipidic membrane [53]. By monitoring spatial changes in M1 dynamics through scanning FCS, they measured a decrease in M1 mobility at the location where membrane curvature was modified and concluded that solely M1-M1 interactions are sufficient to generate lipid membrane curvature. Similar behavior was observed with another strain of Influenza virus (namely, Influenza C Virus), where M1 proteins were able to induce tubular structures on GUVs [54].

In the case of Ebola virus, GUVs were also used to elucidate the interaction of EBOV matrix VP40 with lipids, showing the capacity of VP40 to penetrate into the lipid membrane [55] and to selectively induce vesicles after self-assembly on PS-enriched domains [56].

## References


1. Jouvenet, N.; Bieniasz, P.D.; Simon, S.M. Imaging the Biogenesis of Individual HIV-1 Virions in Live Cells. Nature 2008, 454, 236–240.
2. Floderer, C.; Masson, J.-B.; Boilley, E.; Georgeault, S.; Merida, P.; El Beheiry, M.; Dahan, M.; Roingeard, P.; Sibarita, J.-B.; Favard, C.; et al. Single Molecule Localisation Microscopy Reveals How HIV-1 Gag Proteins Sense Membrane Virus Assembly Sites in Living Host CD4 T Cells. Sci. Rep. 2018, 8, 16283.
3. Michaels, T.C.T.; Bellaiche, M.M.J.; Hagan, M.F.; Knowles, T.P.J. Kinetic Constraints on Self-Assembly into Closed Supramolecular Structures. Sci. Rep. 2017, 7, 12295.
4. Malbec, M.; Sourisseau, M.; Guivel-Benhassine, F.; Porrot, F.; Blanchet, F.; Schwartz, O.; Casartelli, N. HIV-1 Nef Promotes the Localization of Gag to the Cell Membrane and Facilitates Viral Cell-to-Cell Transfer. Retrovirology 2013, 10, 80.
5. Pirrone, G.F.; Emert-Sedlak, L.A.; Wales, T.E.; Smithgall, T.E.; Kent, M.S.; Engen, J.R. Membrane-Associated Conformation of HIV-1 Nef Investigated with Hydrogen Exchange Mass Spectrometry at a Langmuir Monolayer. Anal. Chem. 2015, 87, 7030–7035.

6. Motsa, B.B.; Stahelin, R.V. Lipid–Protein Interactions in Virus Assembly and Budding from the Host Cell Plasma Membrane. Biochem. Soc. Trans. 2021, 49, 1633–1641.
7. Adu-Gyamfi, E.; Soni, S.P.; Xue, Y.; Digman, M.A.; Gratton, E.; Stahelin, R.V. The Ebola Virus Matrix Protein Penetrates into the Plasma Membrane. J. Biol. Chem. 2013, 288, 5779–5789.
8. 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.
9. Kerviel, A.; Thomas, A.; Chaloin, L.; Favard, C.; Muriaux, D. Virus Assembly and Plasma Membrane Domains: Which Came First? Virus Res. 2013, 171, 332–340.
10. 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.
11. 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.
12. 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.
13. Wang, T.; Hong, M. Investigation of the Curvature Induction and Membrane Localization of the Influenza Virus M2 Protein Using Static and Off-Magic-Angle Spinning Solid-State Nuclear Magnetic Resonance of Oriented Bicelles. Biochemistry 2015, 54, 2214–2226.
14. Rossman, J.S.; Lamb, R.A. Influenza Virus Assembly and Budding. Virology 2011, 411, 229–236.
15. Zhou, W.; Resh, M.D. Differential Membrane Binding of the Human Immunodeficiency Virus Type 1 Matrix Protein. J. Virol. 1996, 70, 8540–8548.
16. 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.
17. Ehrlich, L.S.; Fong, S.; Scarlata, S.; Zybarth, G.; Carter, C. Partitioning of HIV-1 Gag and Gag-Related Proteins to Membranes. Biochemistry 1996, 35, 3933–3943.
18. 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)P2/Cholesterol Nanoclusters. Sci. Rep. 2016, 6, 39332.
19. Dick, R.A.; Goh, S.L.; Feigenson, G.W.; Vogt, V.M. HIV-1 Gag Protein Can Sense the Cholesterol and Acyl Chain Environment in Model Membranes. Proc. Natl. Acad. Sci. USA 2012, 109, 18761–

18766.

20. Chukkapalli, 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.
21. Bouamr, F.; Scarlata, S.; Carter, C. Role of Myristylation in HIV-1 Gag Assembly. Biochemistry 2003, 42, 6408–6417.
22. Pérez Socas, L.B.; Ambroggio, E.E. The Influence of Myristoylation, Liposome Surface Charge and Nucleic Acid Interaction in the Partition Properties of HIV-1 Gag-N-Terminal Peptides to Membranes. Biochim. Biophys. Acta Biomembr. 2020, 1862, 183421.
23. Chukkapalli, 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.
24. 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.
25. 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.
26. Keller, H.; Kräusslich, H.-G.; Schwille, P. Multimerizable HIV Gag Derivative Binds to the Liquid-Disordered Phase in Model Membranes. Cell. Microbiol. 2013, 15, 237–247.
27. Urbančič, I.; Brun, J.; Shrestha, D.; Waithe, D.; Eggeling, C.; Chojnacki, J. Lipid Composition but Not Curvature Is the Determinant Factor for the Low Molecular Mobility Observed on the Membrane of Virus-Like Vesicles. Viruses 2018, 10, 415.
28. Gregoriades, A. Interaction of Influenza M Protein with Viral Lipid and Phosphatidylcholine Vesicles. J. Virol. 1980, 36, 470–479.
29. Oxford, J.S.; Hockley, D.J.; Heath, T.D.; Patterson, S. The Interaction of Influenza Virus Haemagglutinin with Phospholipid Vesicles—Morphological and Immunological Studies. J. Gen. Virol. 1981, 52, 329–343.
30. Kerviel, A.; Dash, S.; Moncorgé, O.; Panthu, B.; Prchal, J.; Décimo, 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.
31. 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.

32. 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.
33. 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.
34. Huang, R.T.C.; Warn, K.; Klenk, H.-D.; Rott, R. Association of the Envelope Glycoproteins of Influenza Virus with Liposomes—a Model Study on Viral Envelope Assembly. Virology 1979, 97, 212–217.
35. Bailey, A.; Zhukovsky, M.; Gliozzi, A.; Chernomordik, L.V. Liposome Composition Effects on Lipid Mixing between Cells Expressing Influenza Virus Hemagglutinin and Bound Liposomes. Arch. Biochem. Biophys. 2005, 439, 211–221.
36. 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.
37. Martyna, A.; Gómez-Llobregat, J.; Lindén, M.; Rossman, J.S. Curvature Sensing by a Viral Scission Protein. Biochemistry 2016, 55, 3493–3496.
38. Martyna, A.; Bahsoun, B.; Madsen, J.J.; Jackson, F.S.J.S.; Badham, M.D.; Voth, G.A.; Rossman, J.S. Cholesterol Alters the Orientation and Activity of the Influenza Virus M2 Amphipathic Helix in the Membrane. J. Phys. Chem. B 2020, 124, 6738–6747.
39. Ruigrok, R.W.H.; Schoehn, G.; Dessen, A.; Forest, E.; Volchkov, V.; Dolnik, O.; Klenk, H.-D.; Weissenhorn, W. Structural Characterization and Membrane Binding Properties of the Matrix Protein VP40 of Ebola Virus11Edited by J. Karn. J. Mol. Biol. 2000, 300, 103–112.
40. Scianimanico, S. Membrane Association Induces a Conformational Change in the Ebola Virus Matrix Protein. EMBO J. 2000, 19, 6732–6741.
41. Johnson, K.A.; Taghon, G.J.F.; 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.
42. Valbuena, A.; Maity, S.; Mateu, M.G.; Roos, W.H. Visualization of Single Molecules Building a Viral Capsid Protein Lattice through Stochastic Pathways. ACS Nano 2020, 14, 8724–8734.
43. 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.
44. Miles, P.; Cassidy, P.; Donlon, L.; Yarkoni, O.; Frankel, D. In Vitro Assembly of a Viral Envelope. Soft Matter 2015, 11, 7722–7727.

45. Hilsch, M.; Goldenbogen, B.; Sieben, C.; Höfer, 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.
46. 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.
47. Höfer, 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.
48. Cremer, P.S.; Boxer, S.G. Formation and Spreading of Lipid Bilayers on Planar Glass Supports. J. Phys. Chem. B 1999, 103, 2554–2559.
49. 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.
50. Gui, D.; Gupta, S.; Xu, J.; Zandi, R.; Gill, S.; Huang, I.-C.; Rao, A.L.N.; Mohideen, U. A Novel Minimal in Vitro System for Analyzing HIV-1 Gag-Mediated Budding. J. Biol. Phys. 2015, 41, 135–149.
51. Van Engelenburg, S.B.; Shtengel, G.; Sengupta, P.; Waki, K.; Jarnik, M.; Ablan, S.D.; Freed, E.O.; Hess, H.F.; Lippincott-Schwartz, J. Distribution of ESCRT Machinery at HIV Assembly Sites Reveals Virus Scaffolding of ESCRT Subunits. Science 2014, 343, 653–656.
52. 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.
53. Dahmani, I.; Ludwig, K.; Chiantia, S. Influenza A Matrix Protein M1 Induces Lipid Membrane Deformation via Protein Multimerization. Biosci. Rep. 2019, 39, BSR20191024.
54. Saletti, D.; Radzimanowski, J.; Effantin, G.; Midtvedt, D.; Mangenot, S.; Weissenhorn, W.; Bassereau, P.; Bally, M. The Matrix Protein M1 from Influenza C Virus Induces Tubular Membrane Invaginations in an in Vitro Cell Membrane Model. Sci. Rep. 2017, 7, 40801.
55. Soni, S.P.; Adu-Gyamfi, E.; Yong, S.S.; Jee, C.S.; Stahelin, R.V. The Ebola Virus Matrix Protein Deeply Penetrates the Plasma Membrane: An Important Step in Viral Egress. Biophys. J. 2013, 104, 1940–1949.
56. Soni, S.P.; Stahelin, R.V. The Ebola Virus Matrix Protein VP40 Selectively Induces Vesiculation from Phosphatidylserine-Enriched Membranes. J. Biol. Chem. 2014, 289, 33590–33597.