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Lipid Membrane Interface Viewpoint: From Viral Entry to Antiviral and Vaccine Development

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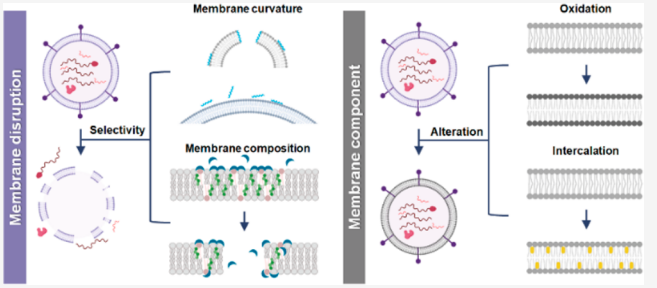
ABSTRACT: Membrane-enveloped viruses are responsible for most viral pandemics in history, and more effort is needed to advance broadly applicable countermeasures to mitigate the impact of future outbreaks. In this Perspective, we discuss how biosensing techniques associated with lipid model membrane platforms are contributing to improving our mechanistic knowledge of membrane fusion and destabilization that is closely linked to viral entry as well as vaccine and antiviral drug development. A key benefit of these platforms is the simplicity of interpreting the results which can be complemented by other techniques to decipher more complicated biological observations and evaluate the biophysical functionalities that can be correlated to biological activities. Then, we introduce exciting application examples of membrane-targeting antivirals that have been refined over time and will continue to improve based on biophysical insights. Two ways to abrogate the function of viral membranes are introduced here: (1) selective disruption of the viral membrane structure and (2) alteration of the membrane component. While both methods are suitable for broadly useful antivirals, the latter also has the potential to produce an inactivated vaccine. Collectively, we emphasize how biosensing tools based on membrane interfacial science can provide valuable information that could be translated into biomedicines and improve their selectivity and performance.



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1. OVERVIEW

To combat unprecedented viral pandemics, global efforts have been expended on vaccine and therapeutic development. While emerging and re-emerging viral pathogens have continuously generated global threats, many circulating viruses that have caused high-profile viral outbreaks are enveloped with lipid bilayers.[1] Examples include coronaviruses (severe acute respiratory syndrome coronaviruses), flaviviruses (Zika, dengue, yellow fever, and West Nile viruses), and influenza viruses and highly fatal ones such as Ebola virus. Although recent successes of lipid-based vaccines and nonpharmacological measures have curbed the health and economic damage, more effort is still needed to advance broadly applicable countermeasures to curtail the impact of future outbreaks.

As the ultimate goal of both viruses and gene delivery systems is to express genes using the target cellular machinery, the core steps in optimizing the gene delivery system resemble those of the viral entry mechanisms (Figure 1). The viral entry of enveloped viruses starts from the binding of the viral entry glycoprotein (e.g., hemagglutinin of influenza virus, gp120/gp41 of HIV, and the spike glycoprotein of Ebola and coronaviruses) to its receptor on host cell membranes. Generally, there are two entry pathways, receptor-mediated endocytosis (endosomal) and direct fusion (cell surface), depending on the site where entry glycoproteins are activated by different pH values, cations, or proteases.[2,3] Although these internalization processes might look distinct, both involve the cleavage or activation of an entry protein to expose the fusion peptide (FP) that can partition into the target membrane (either an endosome or plasma membrane) to trigger virus−cell membrane fusion. Therefore, the interactions between lipid bilayers and FPs have been highlighted to explore how FPs destabilize bilayers, promote negative curvature (for stalk formation), and lower the bilayer rupture tension to eventually lead to the fusion pore, whereby this Perspective also partially covers how biosensing technologies have contributed to this course of study.

Similarly, gene delivery systems such as prophylactic viral vaccines also require effective membrane fusion (i.e., endosomal escape) to maximize protein expression. Indeed, the inactivated whole-virus vaccine can offer immunogenicity; however, the inactivation process quite often damages the antigen structure, resulting in weaker immune responses.[4] Alternatively, a lipid-based gene delivery system such as mRNA-loaded lipid nanoparticles has gained increasing attention by offering unique

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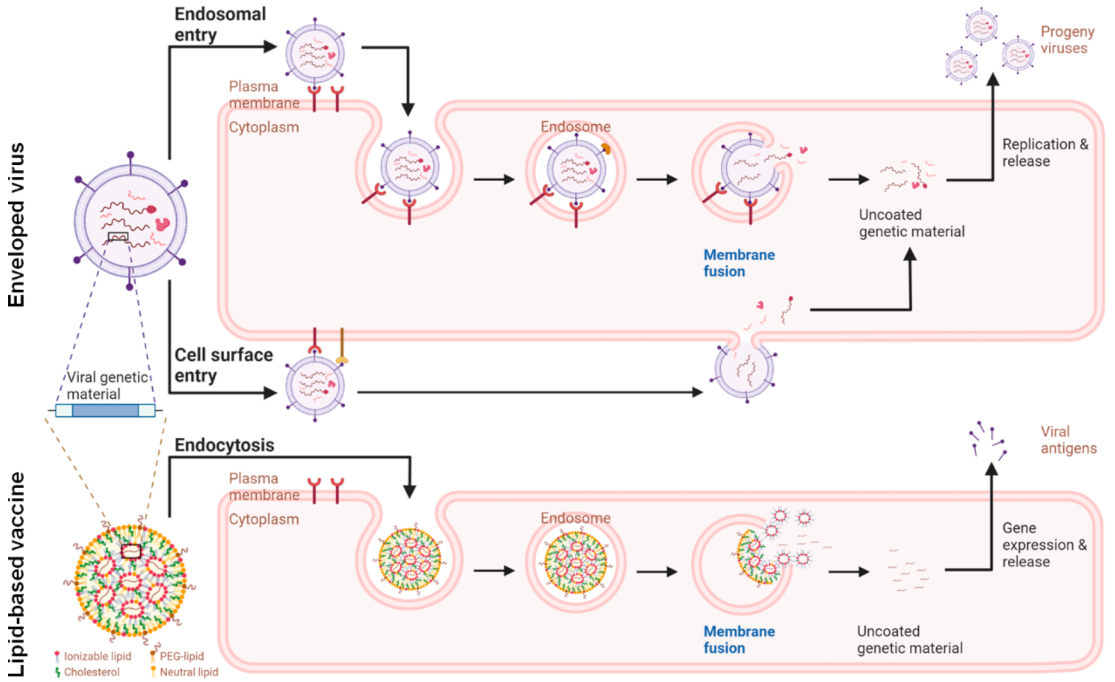

Figure 1. Schematic illustration of entry pathways of enveloped viruses and gene delivery systems with the emphasis of the membrane interfacial aspect of the resemblances between them. In all cases, effective membrane fusion is required to release the uncoated genetic material for further replication or gene expression. For the representative lipid-based vaccine, an mRNA-loaded lipid nanoparticle is presented.

features including cell-free production, the potential for low cost, reproducibility, and relatively higher safety. Lipid nanoparticles are composed of ionizable lipids ($pK_a \approx 6.5$), cholesterol, neutral helper lipids, and poly(ethylene glycol) (PEG)-lipids, which do not encompass other fusion-mediating functional proteins or peptides. Therefore, once lipid nanoparticles are trapped in endosomal compartments by various pathways depending on the cell type and particle itself,[5] the efficient endosomal escape is dependent on the physicochemical properties of lipids. For instance, ionizable lipids from lipid nanoparticles are protonated at low endosomal pH to interact with a negatively charged endosomal membrane, facilitating the fusion to release the genetic material to the cytosol. Although numerous efforts have been made to empirically improve the transfection efficiency by modifying ionizable lipids, there is a great need to employ biophysical characterization tools to corroborate the suggested mechanism and optimize lipid-based gene delivery strategies.

In this Perspective, we will discuss how biosensing techniques associated with various lipid model membrane platforms can contribute to improving the mechanistic knowledge of membrane fusion and destabilization that is closely linked to the viral entry process as well as vaccine and antiviral drug development. A key advantage of these platforms is the simplicity to interpret the results which can be complemented by other techniques to decipher more complicated biological observations and evaluate the biophysical functionalities that can be correlated to biological activities. In addition, we will present exciting classes of antivirals that inhibit enveloped viruses by targeting the lipid membrane of the viruses. As the viral membrane is derived from host cell membranes, this strategy offers a negligible chance for resistance development. There are broadly two ways to inactivate the function of viral membranes: (1) selectively disrupt the viral membrane structure and (2) alter the membrane component while leaving the integrity of the viral particle intact. While both methods are suitable for broadly applicable antiviral treatment, the latter also has the potential to produce an inactivated whole virus vaccine. Furthermore, as the antiviral compounds introduced here are aimed at targeting the viral envelope, an abundance of biophysical characterizations was primarily conducted to examine the mechanisms of action, especially to understand the selectivity for impairing viral rather than host cell membranes. Interestingly, some of them were serendipitously discovered while studying the physicochemical properties of the molecules and have been continuously improved over the past years based on mechanistic details obtained from biophysical characterizations. Collectively, we emphasize how biosensing tools based on membrane interfacial science can provide valuable information that can be translated into antivirals or gene delivery vehicle screening and improvement of their performance.

2. BIOSENSING TECHNIQUES FOR MEMBRANE INTERFACIAL STUDY

In this section, we introduce compelling works of biosensing techniques that were applied to provide mechanistic insights and to advance strategies combating viral infections. The scope of interfacial study here is focused on lipid interactions, especially utilizing lipid model membranes such as lipid vesicles and supported lipid bilayers (SLBs) that mimic the lipid bilayer of enveloped viruses or target host cell membranes. Thus, membrane permeabilization/disruption processes and fusion events are of particular interest among many interactions. Biosensing techniques that are widely used in studying interface characterizations with model membranes are introduced in Table 1.

2.1. Fluorescence-Based Techniques. *2.1.1. Membrane Permeabilization and Lysis.* Fluorescence-based assays are widely used for biophysical profiling to quantitatively compare the membrane permeability/lysis and fusogenicity among

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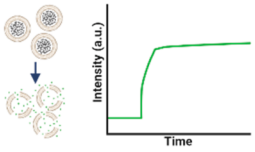
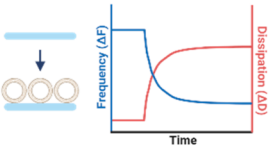
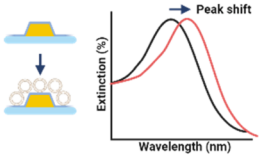
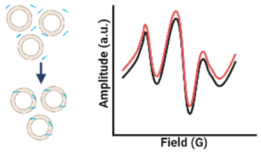
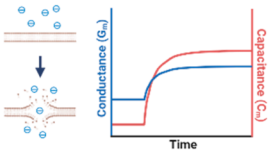
Table 1. Summary of Biosensing Tools to Characterize the Membrane Interface Discussed Here

Technique	Principle	Characterization	Model membrane
Fluorescence spectroscopy/ microscopy		- Fluorescent label, real-time (flow) - Membrane permeabilization/lysis - Morphological change - Membrane fusion - Single-particle level tracking	- Bulk vesicle - Surface adsorbed vesicle - Supported lipid bilayer (SLB) - Tethered vesicle - Tethered bilayer lipid membrane (tBLM)
Quartz crystal microbalance-dissipation (QCM-D)		- Label-free, real-time (flow) - Quantification of wet mass - Penetration depth of 60–250 nm - Viscoelastic properties - Surface-specific kinetic analysis	- Surface adsorbed vesicle - Supported lipid bilayer (SLB) - Tethered bilayer lipid membrane (tBLM)
Localized surface plasmon resonance (LSPR), nanoplasmonic sensors		- Label-free, real-time (flow) - Quantification of dry mass - Decay length of 5–20 nm - Nanohole geometrical diversity - Surface-specific kinetic analysis	- Surface adsorbed vesicle - Supported lipid bilayer (SLB)
Electron spin resonance (ESR) spectroscopy		- Spin label - Membrane organization - Chemical reactions involving radicals	- Bulk vesicle
Electrochemical impedance spectroscopy (EIS)		- Label-free, real-time (flow) - Membrane sealing properties - Membrane destabilization kinetics - Microplate design for high throughput	- Tethered bilayer lipid membrane (tBLM) - Black lipid membrane - Supported lipid bilayer (SLB)

different membrane-active agents. In this context, the extent of membrane disruption induced by membrane-active agents can be potentially translated to their efficacy of impairing viral membranes of multiple enveloped viruses. Therefore, various membrane permeability assays based on model membranes have been utilized and developed not only to screen antiviral candidates' activities but also to investigate underlying mechanisms of membrane disruption. The most accessible platform is based on a bulk lipid vesicle suspension where the probes are either encapsulated or embedded within the lipids. The extent of membrane permeability induced by membrane-active agents can be quantified by either a fluorescence increase (dequenching) or decrease (quenching) (Figure 2A). The amount of fluorescence intensity changes reflecting entrapped dye leakage provides direct comparisons depending on the membrane compositions or various compounds and can offer efficient screening opportunities to evaluate the potencies.[6,7] In order to compensate for the transient permeabilizing effects, two-step assays can be implemented to assess whether the pore formation is permanent or transient after reaching equilibrium.[8]

Although these bulk vesicle suspension-based assays offer valuable findings, there are limitations because the ensemble-averaged result cannot distinguish each occurrence of vesicle disruption involving binding, transient/permanent pore formation, morphological deformation, lysis, and/or fusion. A more sophisticated assay has utilized immobilized vesicles that are doped with entrapped calcein and rhodamine-labeled phospholipid to track membrane permeability and membrane lysis in real time, respectively, whereby the concentrations of both dyes are always below the self-quenching concentration (Figure 2B). Unlike bulk ensemble-averaged measurements, one of the advantages of visualizing at a single-vesicle level is that the simultaneous tracking of pore formation (calcein, green) and membrane disruption (rhodamine, red) kinetics can be analyzed depending on the individual vesicle's diameter (Figure 2C).[9] The introduced membrane-targeting antiviral agent, AH peptide, could induce complete pore formation, followed by membrane lysis which was more efficient when the vesicle size was below 125 nm. Interestingly, the membrane curvature sensing ability could be tuned by incorporating D-enantiomers, whereby the AH-D peptide showed more potent activity than the AH-L peptide by rupturing ∼100% of <150 nm vesicles and exhibited vesicle rupturing activities for up to ∼300 nm vesicles (Figure 2D).[10]

Moreover, multiply labeled giant unilamellar vesicles (GUVs) can be employed to recreate and visualize coexisting lipid phases consisting of raft-like liquid-ordered (L_o) and liquid-disordered (L_d) domains (Figure 2E). Lipid rafts are sphingolipids/cholesterol-enriched domains that are involved in various cellular processes including membrane fusion and viral entry. By incorporating a large amount of sphingomyelin (SM) and cholesterol among unsaturated lipids, GUVs with segregated lipid phases can be prepared. The liquid-disordered region (rich with unsaturated lipids) and the lipid raft region (rich with

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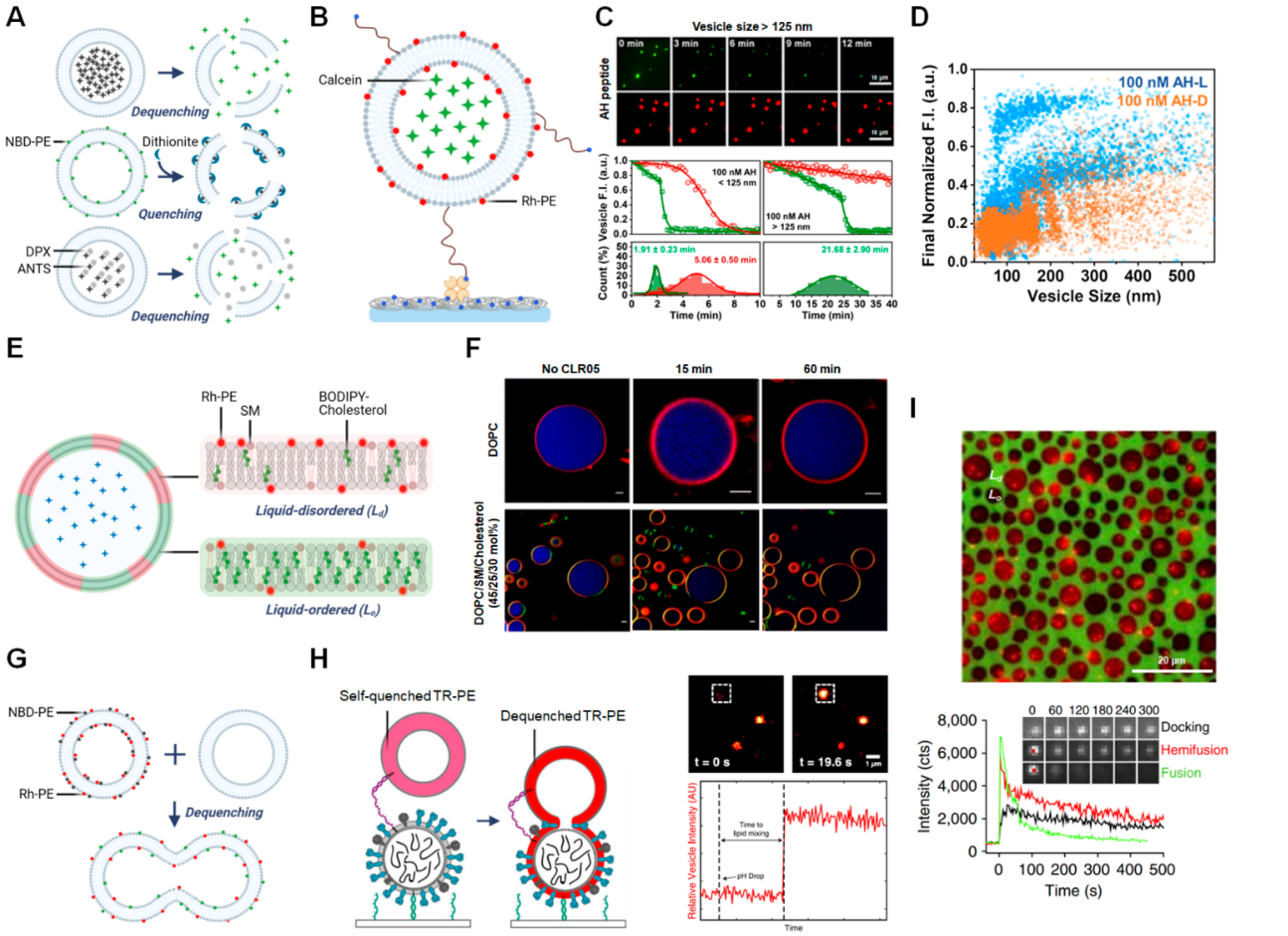

Figure 2. Fluorescence-based techniques that are used in membrane interfacial studies. (A) Bulk liposome suspension-based fluorescence measurements are commonly used in membrane permeabilization assays. (B) Schematic of the dually labeled tethered vesicle platform. (C) Single vesicle assay showing that the AH peptide (L-enantiomer) selectively ruptures vesicles with sizes below 125 nm. (Top) Time-lapse fluorescence micrographs of encapsulated calcein (green) and Rh-PE (red) probes after the addition of 100 nM peptide to large vesicles (>125 nm). (Bottom) Representative kinetic profiles and corresponding histograms of single-vesicle pore formation (green) and lysis (red) kinetics for different vesicle size ranges. Reproduced with permission from ref 9. Copyright 2019 American Chemical Society. (D) Quantification of AH peptide enantiomer-induced vesicle rupture as a function of vesicle diameter, whereby each symbol represents data from an individual vesicle. Reproduced with permission from ref 10. Copyright 2018 Springer Nature. (E) Schematic of GUV with coexisting lipid phases consisting of liquid-disordered (L_d) and raft-like liquid-ordered (L_o) domains. (F) CLR05 selectively disrupts the membrane with L_o domains (bottom: DOPC/SM/cholesterol) but not the L_d domains (top: DOPC). Scale bars are 5 μm. Reproduced with permission from ref 7. Copyright 2020 American Chemical Society. (G) Schematic of the lipid mixing assay using a FRET pair of NBD-PE and Rh-PE. (H) Lipid mixing was detected by surface-tethered virus particles and target membrane vesicles upon lowering the pH. Reproduced with permission from ref 11. Copyright 2020 Elsevier. (I) FP-mediated binding and fusion occur at the interface of L_o (black) and L_d (green) domains. Individual vesicle fusion events with SLBs can be classified by docking, hemifusion, and full fusion events. The scale of all inset images is 2.5 × 2.5 μm^2. Reproduced with permission from ref 12. Copyright 2016 Springer Nature. NBD-PE, nitrobenzoxadiazole-labeled lipid; DPX, *p*-xylene-bis-pyridinium bromide; ANTS, 8-aminonapthalene-1,3,6 trisulfonic acid; Rh-PE, rhodamine-labeled lipid; F.I., fluorescence intensity; TR-PE, Texas red-labeled lipid; SM, sphingomyelin.

cholesterol) can be mainly visualized by red (Rh-PE) and green (BODIPY-cholesterol) channels, respectively, while the entrapped fluorophore molecules can be simultaneously observed by the blue channel. Upon adding a membrane-targeting antiviral agent, CLR05, to the pure DOPC GUV, both the entrapped dye and lipid bilayer have remained largely intact (Figure 2F).[7] However, with the existence of the raft-like domain, CLR05 partially disrupted the membrane integrity. Although the architecture and dimensions of GUVs do not closely mimic those of viral membranes as small unilamellar vesicles might offer, this biophysical result strongly supports the fact that the membrane disruption caused by CLR05 is selective depending on the target membrane lipid composition.

2.1.2. Membrane Fusion. Other than biophysical assays assessing membrane disruption, membrane fusion is another critical event that has been extensively studied using model membranes. It is well known that viruses attach to host cell receptors mediated by the viral glycoprotein, and then the cell entry is led by subunit(s) of glycoprotein to trigger the membrane fusion between them. Therefore, the extent of modeled virus−cell membrane fusion can be correlated with the process of viral entry. Consequently, unraveling the interfacial phenomenon of membrane fusion in relation to the physicochemical environment within a controlled artificial model platform can be translated to the development of strategies to inhibit viral fusion. Furthermore, the extent of fusion between (model) endosomal membranes and nucleic acid-loaded lipid nanoparticles can be correlated with the efficiency of the endosomal escape of genetic materials from the

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lipid carrier, offering biophysical screening opportunities to enhance the transfection efficiency.

The most common assay to evaluate membrane fusion is through lipid mixing, monitoring the redistribution of lipids from the donor to the acceptor membrane. This can be done by using either fluorescence resonance energy transfer (FRET) pairs such as NBD and rhodamine-based lipid-labeled fluorophores or the self-quenching concentration of a marker-labeled lipid (Figure 2G). For example, in order to study the role of FP in membrane fusion, FRET pair-labeled vesicles (quenched) and label-free vesicles can be mixed together with the FP. Then, the FP-mediated fusion can be monitored by the increase in fluorescence intensity caused by the increase in distance between FRET pairs (dequenched).[13] Similar strategies can be applied with labeled endosome-mimicking vesicles and lipid nanoparticles to measure the degree of lipid mixing.[14] However, in bulk measurements, all signals are ensemble-averaged and convolved such that the interpretation of mechanistic details is difficult. Moreover, it should be noted that measuring the extent of lipid mixing in the bulk cannot distinguish among membrane full fusion, hemifusion, and lipid transfer without fusion, thus it requires additional characterization.

To overcome this, later efforts have engaged single-particle-level monitoring of lipid mixing to deconvolve individual vesicles' or virus particles' fusion rate/efficiency and to observe the intermediates such as hemifusion and pore formation before the full membrane merger. While most of the fundamental studies and applications have been established on the basis of lipid−protein interactions,[15,16] enveloped viral particles or FP-bound vesicles were also widely used to detect the kinetics of fusion events.[17−20] Most of the early single-particle fusion assays utilized SLBs as a target membrane and fluorescently marked virus particles with a self-quenching concentration, which required a modification of the viral particles that might affect the activity of envelope proteins.[17,21] More recently, a different configuration of substituting target membrane by vesicles with a self-quenching concentration and immobilizing viral particles requiring less membrane modification has been introduced (Figure 2H).[11] Upon lowering the pH, lipid mixing occurs, and increased fluorescence due to dequenching can be detected on a single-virus scale. By tuning the amount of cholesterol in vesicles, the study found that cholesterol does enhance the (hemi)fusion efficiency by presumably stabilizing the hemifusion state but does not affect the fusion rate. To ensure the fusion event, the target vesicles can be loaded with a self-quenching concentration to monitor the pore formation, and the tether length between the target vesicles and viral particles can be modulated to study the effects of receptor length and flexibility.[22]

Furthermore, phase-separated SLBs can be also applied to study the viral glycoprotein (GP)- or fusion peptide (FP)-mediated binding and fusion that take place at the interface of L_o and L_d. For example, SLBs composed of 60 mol % raft-favoring lipids (e.g., cholesterol, SM, and certain saturated lipids such as DPPC) and 40 mol % unsaturated lipids (e.g., DOPC, DOPS, etc.) labeled with unsaturated phospholipids may produce segregated L_d (green) and L_o (dark) phases (Figure 2I).[23] After HIV FP was preincubated with the fabricated SLBs, vesicles mimicking the viral envelope (red) were added to observe the FP-mediated fusion of single vesicles, which were individually tracked to quantify each possible interaction, namely, docking, hemifusion, and fusion. By varying the membrane composition, it was found that the line tension at the interface of L_o and L_d domain boundaries was required in both target (mimicking the cellular membrane) and vesicle (mimicking the viral particle) membranes to increase the successful fusion rate. Likewise, by utilizing fluorescently labeled model membranes and tracking single-vesicle fusion, FP-mediated fusion could be extensively studied depending on the environmental conditions (e.g., pH and salt concentrations), lipid types, and compositions, which led to valuable insights such as identifying cholesterol-binding motifs in glycoprotein and subsequent countermeasures to lower the viral entry.[24]

2.2. Surface-Specific Kinetics Monitoring. Acoustic- and optical-based surface-sensitive measurements such as quartz crystal microbalance with dissipation monitoring (QCM-D), ellipsometry, and localized surface plasmon resonance (LSPR) have been extensively used to study surface adsorption and binding kinetics. While all techniques offer label-free, real-time detection of surface-specific kinetic analysis, the combination of acoustic and optical approaches is complementary and grants several advantages. First, QCM-D can measure hydrodynamically coupled mass (i.e., acoustic, wet mass) and energy-dissipating (viscoelastic) properties whereas the optical biosensors (ellipsometry and LSPR) can quantify the adsorbate mass (i.e., optical, dry mass). Therefore, by combining both, the hydrodynamically bound solvent (e.g., aqueous water) mass can be deduced in each step of the interaction. For example, when peptide-mediated vesicle destabilization was monitored via simultaneous ellipsometry and QCM-D measurements, the peptide-to-lipid ratio at the moment when peptides bind to adsorbed vesicles could be revealed.[25] Based on concentration-dependent results, it was also discovered that a nearly constant ratio of peptides was required for vesicle rupture, meaning that the peptides' attachment over a critical density would induce the vesicle destabilization. Another interesting example of combining QCM-D and LSPR has utilized their different sensing depths of 60−250 nm and 5−20 nm, respectively, to distinguish the mechanistic steps during the peptide and vesicle interaction, whereby the process of SLB formation could be temporally separated from excess lipid and solvent release.[26] In addition, more recently developed diverse geometries of nanoplasmonic sensor surfaces could enable the sensing of membrane interactions depending on the degrees of membrane curvature.[27] Collectively, surface-sensitive measurements could lay the groundwork for broader interfacial study applications and improvements of functionalities for drug development.

2.3. Membrane Ordering Effect. Electron spin resonance (ESR) spectroscopy can measure the membrane ordering effect using multiple spin-labeled lipids, allowing the determination of the penetration depth of inserted foreign agents such as viral fusion peptides (FPs, N-terminal segment of glycoprotein).[13,28−30] Generally, proper insertion of FPs into the host cell membrane triggers membrane order, thus the viral fusion efficiency could be correlated depending on the depth of FP penetration because the higher lipid ordering value implies the lower-energy barrier required for the virus−membrane fusion. For instance, the changes in membrane ordering parameters depending on the calcium ion concentration with various mutated FP sequences could reveal that FPs indeed induce membrane ordering, and the substitution of negatively charged amino acids for neutral ones reduces the effect.[13] A similar experiment can be also done in a time-resolved manner with lipid vesicles containing other glycoproteins to confirm the membrane ordering effect while docking events between them

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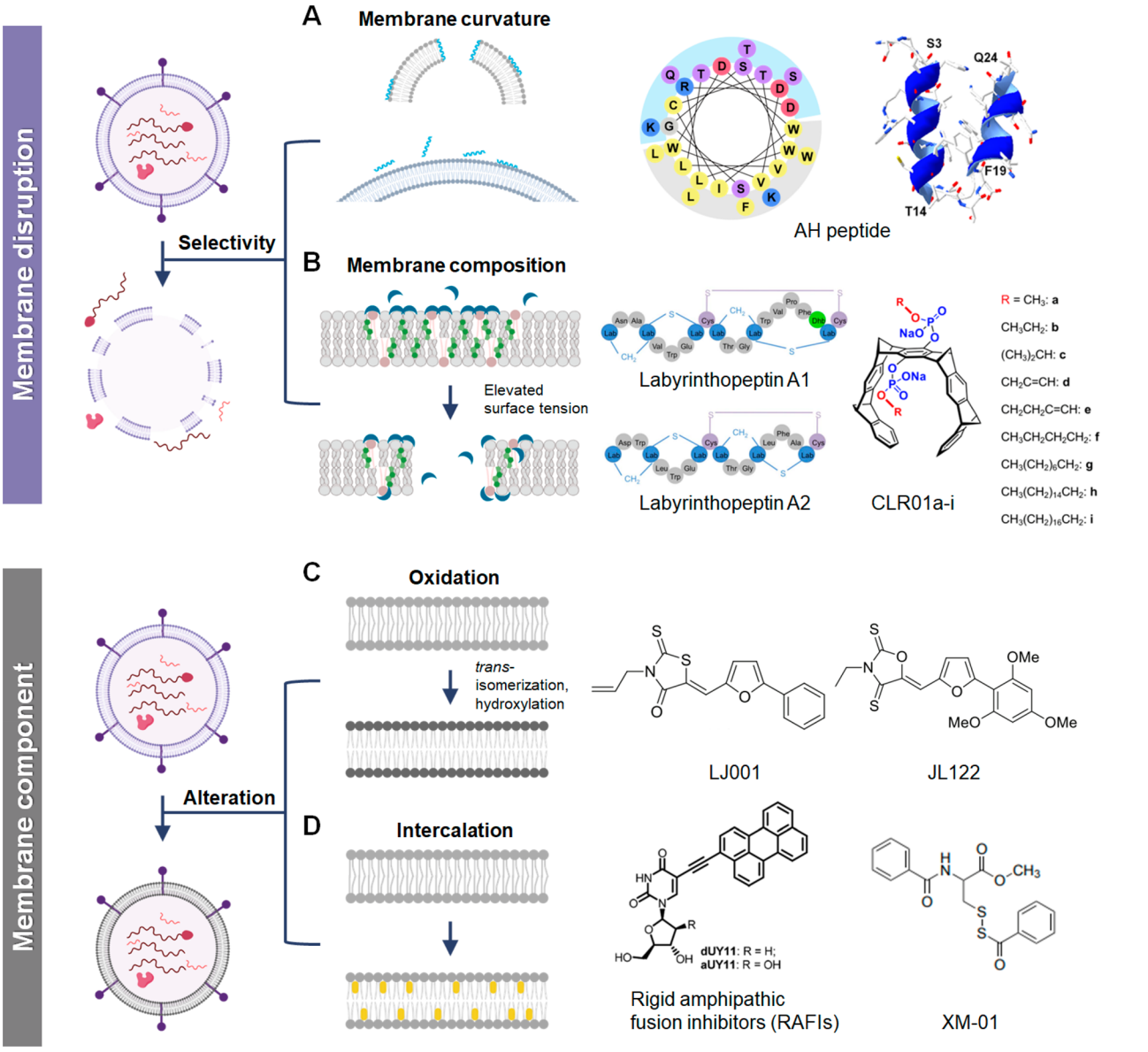

Figure 3. Two ways to abrogate enveloped virus particles: membrane disruption and membrane component alteration. To attain target selectivity for viral envelope disruption, presented antiviral compounds discriminate viral membranes from host cellular membranes by (A) membrane-curvature sensing and (B) membrane composition. To alter the membrane composition and/or fluidity, (C) oxidation (chemical) and (D) intercalation (physical) approaches are displayed. The structure of the AH peptide is reproduced with permission from ref 9. Copyright 2019 American Chemical Society. The structure of labyrinthopeptins A1 and A2 are reproduced with permission from ref 55. Copyright 2013 the authors. The structure of CLR01a-i is reporoduced with permission from ref 7. Copyright 2020 American Chemical Society.

take place. Such an approach has also been proven useful in systematically interpreting biological results on how calcium ion or cholesterol depletion inhibits several types of enveloped virus infections.[31] Combined with circular dichroism spectroscopy, the conformational change in peptides can be also related to the environmental conditions and corresponding membrane ordering effect,[28] leading to the conclusion that the mechanistic interpretation of viral fusion can shed light on the inhibition of viral entry.

2.4. Label-Free Membrane Permeabilization Monitoring. Finally, the tethered bilayer lipid membrane (tBLM) platform coupled with the electrochemical impedance spectroscopy (EIS) technique is introduced to identify types of membrane interaction by monitoring the transmembrane ionic conductance and capacitance. The tBLM offers a more flexible configuration than the SLB due to free aqueous reservoirs existing on both sides of the bilayer and enabling the measurement of the ionic membrane permeability. Depending on how membrane-active compounds partition into and interact with the membrane, EIS measurements can identify mechanisms ranging from the adsorption/aggregation of compounds within membranes to transient/permanent transmembrane pore formation, pore modulation, and the disruption of membranes.[32,33] For instance, human cathelicidin LL-37, known to disrupt the viral envelope,[34] has been shown to have surfactant-like activity (large increases in both conductance and capacitance) whereas α-hemolysin, known as an antibiotic, has displayed pore-forming activity (a large increase only in conductance).[32,35] Similarly, when two antiviral peptides, AH and C5A, have been assessed, the AH peptide disrupted

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membranes via pore formation while the C5A peptide solubilized membranes in a surfactant-like manner, thereby explaining why the C5A peptide exhibited less targeting selectivity.[9] Another recent study utilized the EIS to characterize the membrane disruption of antimicrobial lipids and showed that glycerol monolaurate (GML) induced irreversible pore formation whereas lauric acid (LA) caused reversible membrane damage, which is aligned with the potency that GML has shown over other compounds including LA in antiviral assays.[36] Therefore, there is outstanding potential to implement the EIS associated with tBLM not only to gain mechanistic knowledge of membrane-active compounds but also to screen new compounds and further refine existing ones.

3. TARGETING VIRAL MEMBRANES: BROAD-SPECTRUM TREATMENT TO VACCINE DEVELOPMENT

Viral entry and replication are mediated by distinct binding receptors or enzymes depending on the virus types, thus classical strategies based on the inhibition of specific proteins are quite often limited to a narrow spectrum of viruses and may induce drug-resistant variants. Alternatively, an approach to target the physicochemical properties of viral lipid membranes has emerged to develop versatile antiviral treatments and inactivated vaccines as well. There are broadly two ways to abrogate enveloped virus particles in this approach: by disrupting the structure of the lipid membrane or by changing the lipid composition to obstruct membrane fusion (Figure 3). The former strategy, viral membrane disruption, has been extensively explored to promote selectivity for viral membranes over host cellular membranes. Two approaches to attaining selectivity are discussed here: membrane-curvature sensing and membrane composition. On the other hand, the methods used to alter the membrane composition and/or fluidity are divided into chemical (oxidation) and physical (intercalation) interference, whereby rigid amphipathic fusion inhibitors (RAFIs) are known to exhibit both mechanisms. In addition, a few molecules from the latter strategy have been exploited to generate inactivated enveloped virus vaccines.

3.1. Viral Membrane Disruption. Various compounds ranging from small molecules, fatty acids, and monoglycerides to peptides and proteins can destabilize biological membranes. A common property among these compounds is sufficiently high hydrophobicity and amphipathicity to gain the propensity to insert into membranes, which is termed interfacial activity.[37] Numerous compounds, especially cationic host defense peptides, have been reported to show *in vitro* broad-spectrum antiviral activities; however, the proposed mechanisms of action are quite often diverse, and sometimes they also act against nonenveloped viruses.[38] To simplify the complicated biological results, model membranes with biosensing measurements are powerful tools for interrogating how membrane-active agents affect the integrity of the lipid structure. One of the exemplary works has proven that a library of interfacially active peptides indeed showed inhibitory activity against enveloped viruses due to their ability to interact with virions and destabilize the viral membrane.[39] Accordingly, the next most important aspect to consider is the selectivity for the virus rather than the host cell, and then the understanding of this mechanism should be followed to further improve the function of antivirals. In this section, several antivirals whose mechanism of selectivity has been relatively well established are introduced.

The first category is curvature-dependent membrane disruption by certain antiviral peptides, represented by α-helical (AH) peptides, which are one of the most diversely studied in biophysical perspectives among membrane-targeting drug candidates (Figure 3A). The AH peptide, derived from hepatitis C virus (HCV) nonstructural 5A (NS5A) protein, was empirically discovered to effectively rupture lipid vesicles below a certain diameter while studying membrane interactions that potentially might influence NS5A membrane associations.[40−44] The follow-up studies using biosensing tools have demonstrated that this membrane-disruptive behavior was related to the membrane curvature-dependent pore formation and resulting strain-induced membrane lysis.[43−46] Also, the AH peptide with a net charge of 0 has been shown to disrupt lipid membranes independent of membrane compositions (surface charges[47] or cholesterol[48]). The engineered D-amino acid variant of the AH peptide has been shown to work *in vivo* to therapeutically treat lethal Zika virus infection in mice by reducing viral loads and blunting virus-related inflammation, and the peptide was also able to cross the blood−brain barrier.[10] Recent molecular dynamics simulations have shown that the AH peptide more preferentially binds to stretched membranes where lipid packing defects are exposed and induces stable pore formation in a tetrameric placement.[49]

A shorter analog of the AH peptide with conserved mutations, C5A peptide, is another antiviral peptide that was discovered through high-throughput screening while investigating the potential anti-HCV activity.[50] Early works have shown that the C5A peptide exerts potent antiviral activity against numerous enveloped viruses; however, it was also reported that the C5A peptide exhibits greater cytotoxicity than the AH peptide, thus topical microbicide applications were proposed.[50−54] From a mechanistic viewpoint, an important distinction between the AH and C5A peptides is that the C5A peptide exhibits less targeting selectivity possibly due to its more pronounced coil-to-helix transitions regardless of membrane curvature.[51,54] Taken together, such functional differences caused by conformation changes depending on the peptide length among conserved mutations motivate a deeper investigation of the membrane-interaction profiles.

The next interesting strategy involves a selective disruption depending on the lipid composition (Figure 3B). The first example is labyrinthopeptins (A1 and A2) which belong to a class of polycyclic peptide antibiotics produced by bacteria (lantibiotics), partially composed of post-translationally modified amino acids. After the broad antiviral activity of labyrinthopeptins against enveloped viruses was biologically established,[55] further studies utilized simple yet straightforward biophysical assays involving model membranes to identify a molecular target.[6,56] By monitoring lipid binding and *in vitro* dose−response assays, it was confirmed that both labyrinthopeptins preferentially bound to the ethanolamine headgroup of glycerophospholipids and their antiviral effects were indeed suppressed by the presence of PE-containing liposomes, respectively.[6] Furthermore, the carboxyfluorescein leakage assay delineated that both labyrinthopeptins not only bind to PE but also disrupt the membrane integrity, while their activities can be synergistic and more pronounced within the membrane lipid raft compositions. A similar approach has been utilized in establishing a mechanism of action of cationic CPXV012 peptide (derived from cowpox virus protein) that directly interacts with negatively charged phosphatidylserine in the viral envelope.[57]

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Another class is termed supramolecular ligands or tweezers, CLR01, and acts mainly by disrupting lipid raft-rich domains of the lipid membrane. CLR01 was developed for a lysine- and arginine-specific ligand and was originally applied to inhibit noncovalent molecular interactions during the abnormal self-assembly of proteins (e.g., amyloid aggregates).[58] After observing the unexpected virucidal effect of CLR01, the model membrane permeability assay offered direct proof that a subset of these molecular tweezers preferentially bind to the rigid lipid raft domain, rich with sphingolipid and cholesterol, and disrupt the membrane integrity.[59] A recent study further unraveled that the CLR01 molecule inserts into phosphate groups of the outer membrane leaflet, forming a more stable complex with sphingomyelin rather than with phosphocholine (PC) lipids.[7] This supramolecular docking increases the membrane surface tension, leading to an eventual membrane disruption, which is a more susceptible event in viral membranes than in host cell membranes due to the much higher contents of lipid rafts in viral membranes. This insight might also explain the observation above where labyrinthopeptins induced greater leakage in the lipid raft composition compared to liquid-disordered vesicles when the PE amounts were equal. Furthermore, a series of advanced tweezers with additional aliphatic anchors were developed, showing significantly enhanced biophysical and biological activities, which sets another great example of how rational design based on established mechanistic details may accelerate antiviral therapy development.

3.2. Viral Membrane Component Alteration. The next group of antivirals includes photosensitizing agents that can alter the membrane component by mainly oxidizing unsaturated phospholipids to change the membrane fluidity (Figure 3C). The first generation of this group is LJ001, showing potent *in vitro* antiviral activity against various enveloped viruses.[60] However, as a photosensitizing agent, this molecule is light-dependent and requires oxygen for its activity, thus the application of LJ001 was pivoted to aquaculture environments. For instance, the infectivity of the LJ001-preincubated fish virus (infectious hematopoietic necrosis virus, IHNV) was largely reduced *in vitro* and *in vivo* (in rainbow trout). Furthermore, LJ001-inactivated IHNV showed appreciable immunogenicity, suggesting the potential use of this approach in vaccine applications.[61]

In addition to chemically altering membrane components, there is a physical way to prevent virus−cell fusion (Figure 3D). A good example that acts by mechanical incorporation and subsequent alteration of the membrane curvature is a class of rigid amphipathic fusion inhibitors (RAFIs).[62] As the rigid, planar hydrophobic domain of RAFIs was derived from perylene, a well-known photosensitizer, it had been unclear whether their antiviral efficacy is attributed to photogenerated singlet oxygen (similar to the LJ001 series) or the mechanical alteration of the membrane curvature or an interplay of both effects (which might involve other influences instead of the lipid membrane).[63] Nevertheless, a recent study has screened RAFI analogs using fluorescence spectral measurements with model membranes and has shown that the biophysical properties (e.g., molecular geometry, rigidity, and amphipathicity) rather than certain chemical functional groups of RAFIs were highly responsible for triggering intercalation, which is a prerequisite for inactivating enveloped viruses in this group.[64]

There are also certain hydrogen sulfide (H_2S) donors that to intercalate into viral membranes to inhibit infection. Initially, the mechanism of H_2S-releasing molecules such as GYY4137 has been focused on biological effects, mainly to evaluate the role of H_2S in several paramyxovirus infections.[65] Then, a series of biological assays confirmed that the GYY4137 treatment inhibits the virus−cell fusion process without influencing the viral genome or protein; however, a clear mechanism of how this molecule or H_2S affects the viral membrane had remained unclear.[66] Later, in the course of developing controllable hydrogen sulfide release donors, a new class of cysteine-based molecules, the XM series, was discovered to show antiviral efficacy against enveloped viruses.[67] Recently, one of the mechanisms of action was identified via ESR that XM-01 intercalates into the hydrophobic domain of lipid bilayers and increases the membrane order, leading to the inhibition of virus−cell membrane fusion while leaving the viral glycoproteins and genomes largely unaffected.[68] As mentioned above, XM-01 was applied to generate an inactivated influenza virus for vaccine development that has the potential to produce multiple types of enveloped virus vaccines in a timely manner.

4. CONCLUSIONS AND OUTLOOK

Despite the recent progress of lipid-based vaccines, there is an outstanding need to further improve the delivery efficacy and develop alternative antiviral strategies to prepare for future disease X outbreaks that might be caused by newly emerging viruses and variants of enveloped viruses. In the life cycle of enveloped viruses, effective virus−cell membrane fusion is critical to releasing uncoated genetic materials into the cytoplasm for gene expression and replication. By studying these biological phenomena using biosensing tools associated with lipid model membranes, it is possible to not only decipher complicated mechanisms involving membrane−protein environments but also obtain valuable insights to develop antiviral strategies and gene delivery systems.

Within this scope, we introduced exciting applications of biosensing technologies to study lipid membrane interface interactions, particularly membrane fusion (to correlate efficient viral entry and gene delivery) and membrane destabilization (to correlate the potency and selectivity of antivirals). Broadly, four types of biosensing tools have been discussed: (1) fluorescence-based assays to evaluate permeability and fusion using single-particle-level profilings, GUVs, and SLBs including coexisting lipid phases; (2) acoustic- and optical-based sensors for surface-sensitive measurements to quantify biomolecular mass and distinguish the mechanistic steps; (3) ESR spectroscopy to study the membrane ordering effect caused by FPs or other membrane-intercalating agents; and (4) EIS coupled with tBLM to identify the types of membrane interaction and evaluate the membrane permeability.

Finally, by addressing the latest works of various classes of membrane-targeting agents for broadly applicable antiviral agents, we emphasize that they have been refined over the past years based on the efforts to understand membrane-related mechanisms of action using various biophysical characterizations. Importantly, this approach is not confined to the antiviral field but extends to combat other microorganisms that cause infectious diseases. Furthermore, there are many other underexplored lipid-membrane-targeting compounds ranging from interfacially active peptides including various host defense peptides,[38] antimicrobial peptoids,[69] and analogs of the AH peptide[70] to photosensitizers[71] and H_2S donors[66] that potentially act against enveloped viruses in a more potent and selective way. In addition, there are numerous possibilities for exploiting model membranes to improve the delivery efficacy of

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lipid-based gene delivery systems based on biophysical characterizations[72] and nanoarchitecture design strategies,[73] which can be done by adapting and extending the existing frameworks built on membrane interface research.

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Notes

The authors declare the following competing financial interest(s): N.-J.C. is a co-inventor on US patent no. 8,728,793 that is related to the application of AH peptide molecules for antiviral therapy.

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REFERENCES

(1) Yoon, B. K.; Jeon, W.-Y.; Sut, T. N.; Cho, N.-J.; Jackman, J. A. Stopping membrane-enveloped viruses with nanotechnology strategies: Toward antiviral drug development and pandemic preparedness. *ACS Nano* **2021**, *15* (1), 125−148.

(2) Ryu, W.-S. Virus life cycle. *Molecular Virology of Human Pathogenic Viruses* **2017**, 31−45.

(3) Jackson, C. B.; Farzan, M.; Chen, B.; Choe, H. Mechanisms of SARS-CoV-2 entry into cells. *Nat. Rev. Mol. Cell Biol.* **2022**, *23* (1), 3−20.

(4) Delrue, I.; Verzele, D.; Madder, A.; Nauwynck, H. J. Inactivated virus vaccines from chemistry to prophylaxis: merits, risks and challenges. *Expert Review of Vaccines* **2012**, *11* (6), 695−719.

(5) Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. *Nature Reviews Materials* **2021**, *6* (12), 1078−1094.

(6) Prochnow, H.; Rox, K.; Birudukota, N. S.; Weichert, L.; Hotop, S.-K.; Klahn, P.; Mohr, K.; Franz, S.; Banda, D. H.; Blockus, S. Labyrinthopeptins exert broad-spectrum antiviral activity through lipid-binding-mediated virolysis. *Journal of Virology* **2020**, *94* (2), No. e01471-19.

(7) Weil, T.; Groß, R. d.; Röcker, A.; Bravo-Rodriguez, K.; Heid, C.; Sowislok, A.; Le, M.-H.; Erwin, N.; Dwivedi, M.; Bart, S. M. Supramolecular mechanism of viral envelope disruption by molecular tweezers. *J. Am. Chem. Soc.* **2020**, *142* (40), 17024−17038.

(8) Krauson, A. J.; He, J.; Wimley, W. C. Gain-of-function analogues of the pore-forming peptide melittin selected by orthogonal high-throughput screening. *J. Am. Chem. Soc.* **2012**, *134* (30), 12732−12741.

(9) Park, S.; Jackman, J. A.; Cho, N.-J. Comparing the membrane-interaction profiles of two antiviral peptides: Insights into structure-function relationship. *Langmuir* **2019**, *35* (30), 9934−9943.

(10) Jackman, J. A.; Costa, V. V.; Park, S.; Real, A. L. C.; Park, J. H.; Cardozo, P. L.; Ferhan, A. R.; Olmo, I. G.; Moreira, T. P.; Bambirra, J. L. Therapeutic treatment of Zika virus infection using a brain-penetrating antiviral peptide. *Nat. Mater.* **2018**, *17* (11), 971−977.

(11) Liu, K. N.; Boxer, S. G. Target membrane cholesterol modulates single influenza virus membrane fusion efficiency but not rate. *Biophys. J.* **2020**, *118* (10), 2426−2433.

(12) Yang, S.-T.; Kiessling, V.; Tamm, L. K. Line tension at lipid phase boundaries as driving force for HIV fusion peptide-mediated fusion. *Nat. Commun.* **2016**, *7* (1), 11401.

(13) Nathan, L.; Lai, A. L.; Millet, J. K.; Straus, M. R.; Freed, J. H.; Whittaker, G. R.; Daniel, S. Calcium ions directly interact with the ebola virus fusion peptide to promote structure-function changes that enhance infection. *ACS Infectious Diseases* **2020**, *6* (2), 250−260.

(14) Lee, S. M.; Cheng, Q.; Yu, X.; Liu, S.; Johnson, L. T.; Siegwart, D. J. A systematic study of unsaturation in lipid nanoparticles leads to improved mRNA transfection in vivo. *Angew. Chem.* **2021**, *133* (11), 5912−5917.

https://doi.org/10.1021/acs.langmuir.2c02501
Langmuir XXXX, XXX, XXX−XXX

(15) Yoon, T.-Y.; Okumus, B.; Zhang, F.; Shin, Y.-K.; Ha, T. Multiple intermediates in SNARE-induced membrane fusion. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (52), 19731−19736.
(16) Christensen, S. M.; Stamou, D. G. Sensing-applications of surface-based single vesicle arrays. *Sensors* **2010**, *10* (12), 11352−11368.
(17) Wessels, L.; Elting, M. W.; Scimeca, D.; Weninger, K. Rapid membrane fusion of individual virus particles with supported lipid bilayers. *Biophys. J.* **2007**, *93* (2), 526−538.
(18) Floyd, D. L.; Ragains, J. R.; Skehel, J. J.; Harrison, S. C.; Van Oijen, A. M. Single-particle kinetics of influenza virus membrane fusion. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (40), 15382−15387.
(19) Costello, D. A.; Lee, D. W.; Drewes, J.; Vasquez, K. A.; Kisler, K.; Wiesner, U.; Pollack, L.; Whittaker, G. R.; Daniel, S. Influenza virus-membrane fusion triggered by proton uncaging for single particle studies of fusion kinetics. *Anal. Chem.* **2012**, *84* (20), 8480−8489.
(20) Otterstrom, J. J.; Brandenburg, B.; Koldijk, M. H.; Juraszek, J.; Tang, C.; Mashaghi, S.; Kwaks, T.; Goudsmit, J.; Vogels, R.; Friesen, R. H. Relating influenza virus membrane fusion kinetics to stoichiometry of neutralizing antibodies at the single-particle level. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (48), E5143−E5148.
(21) Niles, W. D.; Cohen, F. S. Fusion of influenza virions with a planar lipid membrane detected by video fluorescence microscopy. *J. Gen. Physiol.* **1991**, *97* (6), 1101−1119.
(22) Webster, E. R.; Liu, K. N.; Rawle, R. J.; Boxer, S. G. Modulating the Influenza A Virus-Target Membrane Fusion Interface With Synthetic DNA-Lipid Receptors. *Langmuir* **2022**, *38* (7), 2354−2362.
(23) Yang, S.-T.; Kiessling, V.; Simmons, J. A.; White, J. M.; Tamm, L. K. HIV gp41-mediated membrane fusion occurs at edges of cholesterol-rich lipid domains. *Nat. Chem. Biol.* **2015**, *11* (6), 424.
(24) Lee, J.; Kreutzberger, A. J.; Odongo, L.; Nelson, E. A.; Nyenhuis, D. A.; Kiessling, V.; Liang, B.; Cafiso, D. S.; White, J. M.; Tamm, L. K. Ebola virus glycoprotein interacts with cholesterol to enhance membrane fusion and cell entry. *Nat. Struct. Mol. Biol.* **2021**, *28* (2), 181−189.
(25) Jackman, J. A.; Goh, H. Z.; Zhdanov, V. P.; Knoll, W.; Cho, N.-J. Deciphering how pore formation causes strain-induced membrane lysis of lipid vesicles. *J. Am. Chem. Soc.* **2016**, *138* (4), 1406−1413.
(26) Ferhan, A. R.; Jackman, J. A.; Cho, N.-J. Integration of quartz crystal microbalance-dissipation and reflection-mode localized surface plasmon resonance sensors for biomacromolecular interaction analysis. *Anal. Chem.* **2016**, *88* (24), 12524−12531.
(27) Ferhan, A. R.; Jackman, J. A.; Malekian, B.; Xiong, K.; Emilsson, G.; Park, S.; Dahlin, A. B.; Cho, N.-J. Nanoplasmonic sensing architectures for decoding membrane curvature-dependent biomacromolecular interactions. *Anal. Chem.* **2018**, *90* (12), 7458−7466.
(28) Lai, A. L.; Freed, J. H. HIV gp41 fusion peptide increases membrane ordering in a cholesterol-dependent fashion. *Biophys. J.* **2014**, *106* (1), 172−181.
(29) Lai, A. L.; Millet, J. K.; Daniel, S.; Freed, J. H.; Whittaker, G. R. The SARS-CoV fusion peptide forms an extended bipartite fusion platform that perturbs membrane order in a calcium-dependent manner. *J. Mol. Biol.* **2017**, *429* (24), 3875−3892.
(30) Ge, M.; Freed, J. H. Fusion peptide from influenza hemagglutinin increases membrane surface order: an electron-spin resonance study. *Biophys. J.* **2009**, *96* (12), 4925−4934.
(31) Liao, Z.; Cimakasky, L. M.; Hampton, R.; Nguyen, D. H.; Hildreth, J. E. Lipid rafts and HIV pathogenesis: host membrane cholesterol is required for infection by HIV type 1. *Biochimi. Biophys. Acta (BBA)-Biomembranes* **2004**, *1863* (11), 1009−1019.
(32) Alghalayini, A.; Garcia, A.; Berry, T.; Cranfield, C. G. The use of tethered bilayer lipid membranes to identify the mechanisms of antimicrobial peptide interactions with lipid bilayers. *Antibiotics* **2019**, *8* (1), 12.
(33) Nikoleli, G.-P.; Siontorou, C. G.; Nikolelis, M.-T.; Bratakou, S.; Bendos, D. K. Recent lipid membrane-based biosensing platforms. *Applied Sciences* **2019**, *9* (9), 1745.
(34) Tripathi, S.; Tecle, T.; Verma, A.; Crouch, E.; White, M.; Hartshorn, K. L. The human cathelicidin LL-37 inhibits influenza A viruses through a mechanism distinct from that of surfactant protein D or defensins. *Journal of General Virology* **2013**, *94* (1), 40.
(35) Cranfield, C. G.; Cornell, B. A.; Grage, S. L.; Duckworth, P.; Carne, S.; Ulrich, A. S.; Martinac, B. Transient potential gradients and impedance measures of tethered bilayer lipid membranes: Pore-forming peptide insertion and the effect of electroporation. *Biophys. J.* **2014**, *106* (1), 182−189.
(36) Jackman, J. A.; Hakobyan, A.; Zakaryan, H.; Elrod, C. C. Inhibition of African swine fever virus in liquid and feed by medium-chain fatty acids and glycerol monolaurate. *Journal of Animal Science and Biotechnology* **2020**, *11* (1), 114.
(37) Rathinakumar, R.; Walkenhorst, W. F.; Wimley, W. C. Broad-spectrum antimicrobial peptides by rational combinatorial design and high-throughput screening: the importance of interfacial activity. *J. Am. Chem. Soc.* **2009**, *131* (22), 7609−7617.
(38) Mookherjee, N.; Anderson, M. A.; Haagsman, H. P.; Davidson, D. J. Antimicrobial host defence peptides: functions and clinical potential. *Nat. Rev. Drug Discovery* **2020**, *19* (5), 311−332.
(39) Hoffmann, A. R.; Guha, S.; Wu, E.; Ghimire, J.; Wang, Y.; He, J.; Garry, R. F.; Wimley, W. C. Broad-spectrum antiviral entry inhibition by interfacially active peptides. *Journal of Virology* **2020**, *94* (23), No. e01682-20.
(40) Cho, N.-J.; Cheong, K. H.; Lee, C.; Frank, C. W.; Glenn, J. S. Binding Dynamics of Hepatitis C Virus' NS5A Amphipathic Peptide to Cell and Model Membranes. *J. Virol.* **2007**, *81* (12), 6682−6689.
(41) Jackman, J. A.; Cho, N.-J. Model Membrane Platforms for Biomedicine: Case Study on Antiviral Drug Development. *Biointerphases* **2012**, *7* (1), 18.
(42) Cho, N.-J.; Lee, C.; Pang, P. S.; Pham, E. A.; Fram, B.; Nguyen, K.; Xiong, A.; Sklan, E. H.; Elazar, M.; Koytak, E. S. Phosphatidylinositol 4, 5-Bisphosphate is an HCV NS5A Ligand and Mediates Replication of the Viral Genome. *Gastroenterology* **2015**, *148* (3), 616−625.
(43) Cho, N.-J.; Dvory-Sobol, H.; Xiong, A.; Cho, S.-J.; Frank, C. W.; Glenn, J. S. Mechanism of an Amphipathic α-Helical Peptide's Antiviral Activity Involves Size-Dependent Virus Particle Lysis. *ACS Chem. Biol.* **2009**, *4* (12), 1061−1067.
(44) Jackman, J. A.; Zan, G. H.; Zhdanov, V. P.; Cho, N.-J. Rupture of Lipid Vesicles by a Broad-Spectrum Antiviral Peptide: Influence of Vesicle Size. *J. Phys. Chem. B* **2013**, *117* (50), 16117−16128.
(45) Cho, N.-J.; Cho, S.-J.; Cheong, K. H.; Glenn, J. S.; Frank, C. W. Employing an Amphipathic Viral Peptide to Create a Lipid Bilayer on Au and TiO2. *J. Am. Chem. Soc.* **2007**, *129* (33), 10050−10051.
(46) Jackman, J. A.; Goh, H. Z.; Zhdanov, V. P.; Knoll, W.; Cho, N.-J. Deciphering How Pore Formation Causes Strain-Induced Membrane Lysis of Lipid Vesicles. *J. Am. Chem. Soc.* **2016**, *138* (4), 1406−1413.
(47) Zan, G. H.; Jackman, J. A.; Cho, N.-J. AH Peptide-Mediated Formation of Charged Planar Lipid Bilayers. *J. Phys. Chem. B* **2014**, *118* (13), 3616−3621.
(48) Park, S.; Jackman, J. A.; Cho, N.-J. Quantitative accounting of dye leakage and photobleaching in single lipid vesicle measurements: Implications for biomacromolecular interaction analysis. *Colloids Surf., B* **2019**, *182*, 110338.
(49) Chng, C.-P.; Cho, N.-J.; Hsia, K. J.; Huang, C. Role of Membrane Stretch in Adsorption of Antiviral Peptides onto Lipid Membranes and Membrane Pore Formation. *Langmuir* **2021**, *37* (45), 13390−13398.
(50) Cheng, G.; Montero, A.; Gastaminza, P.; Whitten-Bauer, C.; Wieland, S. F.; Isogawa, M.; Fredericksen, B.; Selvarajah, S.; Gallay, P. A.; Ghadiri, M. R.; Chisari, F. V. A Virocidal Amphipathic α-Helical Peptide that Inhibits Hepatitis C Virus Infection In Vitro. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (8), 3088−3093.
(51) Jackman, J. A.; Shi, P.-Y.; Cho, N.-J. Targeting the Achilles Heel of Mosquito-Borne Viruses for Antiviral Therapy. *ACS Infect. Dis.* **2019**, *5* (1), 4−8.
(52) Bobardt, M. D.; Cheng, G.; de Witte, L.; Selvarajah, S.; Chatterji, U.; Sanders-Beer, B. E.; Geijtenbeek, T. B.; Chisari, F. V.; Gallay, P. A. Hepatitis C virus NS5A Anchor Peptide Disrupts Human Immunodeficiency Virus. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (14), 5525−5530.

https://doi.org/10.1021/acs.langmuir.2c02501
Langmuir XXXX, XXX, XXX−XXX

(53) de Witte, L.; Bobardt, M. D.; Chatterji, U.; van Loenen, F. B.; Verjans, G. M.; Geijtenbeek, T. B.; Gallay, P. A. HSV neutralization by the microbicidal candidate C5A. *PloS One* **2011**, *6* (5), No. e18917.
(54) Jackman, J. A.; Saravanan, R.; Zhang, Y.; Tabaei, S. R.; Cho, N. J. Correlation between membrane partitioning and functional activity in a single lipid vesicle assay establishes design guidelines for antiviral peptides. *Small* **2015**, *11* (20), 2372−2379.
(55) Férir, G.; Petrova, M. I.; Andrei, G.; Huskens, D.; Hoorelbeke, B.; Snoeck, R.; Vanderleyden, J.; Balzarini, J.; Bartoschek, S.; Brönstrup, M. The lantibiotic peptide labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. *PloS one* **2013**, *8* (5), No. e64010.
(56) Blockus, S.; Sake, S. M.; Wetzke, M.; Grethe, C.; Graalmann, T.; Pils, M.; Le Goffic, R.; Galloux, M.; Prochnow, H.; Rox, K. Labyrinthopeptins as virolytic inhibitors of respiratory syncytial virus cell entry. *Antiviral Res.* **2020**, *177*, 104774.
(57) Luteijn, R. D.; Praest, P.; Thiele, F.; Sadasivam, S. M.; Singethan, K.; Drijfhout, J. W.; Bach, C.; de Boer, S. M.; Lebbink, R. J.; Tao, S. A broad-spectrum antiviral peptide blocks infection of viruses by binding to phosphatidylserine in the viral envelope. *Cells* **2020**, *9* (9), 1989.
(58) Schrader, T.; Bitan, G.; Klärner, F.-G. Molecular tweezers for lysine and arginine-powerful inhibitors of pathologic protein aggregation. *Chem. Commun.* **2016**, *52* (76), 11318−11334.
(59) Lump, E.; Castellano, L. M.; Meier, C.; Seeliger, J.; Erwin, N.; Sperlich, B.; Stürzel, C. M.; Usmani, S.; Hammond, R. M.; von Einem, J. A molecular tweezer antagonizes seminal amyloids and HIV infection. *Elife* **2015**, *4*, e05397.
(60) Wolf, M. C.; Freiberg, A. N.; Zhang, T.; Akyol-Ataman, Z.; Grock, A.; Hong, P. W.; Li, J.; Watson, N. F.; Fang, A. Q.; Aguilar, H. C. A broad-spectrum antiviral targeting entry of enveloped viruses. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (7), 3157−3162.
(61) Balmer, B. F.; Powers, R. L.; Zhang, T.-H.; Lee, J.; Vigant, F.; Lee, B.; Jung, M. E.; Purcell, M. K.; Snekvik, K.; Aguilar, H. C. Inhibition of an aquatic rhabdovirus demonstrates promise of a broad-spectrum antiviral for use in aquaculture. *Journal of Virology* **2017**, *91* (4), No. e02181-16.
(62) St. Vincent, M. R.; Colpitts, C. C.; Ustinov, A. V.; Muqadas, M.; Joyce, M. A.; Barsby, N. L.; Epand, R. F.; Epand, R. M.; Khramyshev, S. A.; Valueva, O. A. Rigid amphipathic fusion inhibitors, small molecule antiviral compounds against enveloped viruses. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (40), 17339−17344.
(63) Vigant, F.; Hollmann, A.; Lee, J.; Santos, N. C.; Jung, M. E.; Lee, B. The rigid amphipathic fusion inhibitor dUY11 acts through photosensitization of viruses. *Journal of Virology* **2014**, *88* (3), 1849−1853.
(64) Speerstra, S.; Chistov, A. A.; Proskurin, G. V.; Aralov, A. V.; Ulashchik, E. A.; Streshnev, P. P.; Shmanai, V. V.; Korshun, V. A.; Schang, L. M. Antivirals acting on viral envelopes via biophysical mechanisms of action. *Antiviral Res.* **2018**, *149*, 164−173.
(65) Li, H.; Ma, Y.; Escaffre, O.; Ivanciuc, T.; Komaravelli, N.; Kelley, J. P.; Coletta, C.; Szabo, C.; Rockx, B.; Garofalo, R. P. Role of hydrogen sulfide in paramyxovirus infections. *Journal of Virology* **2015**, *89* (10), 5557−5568.
(66) Citi, V.; Martelli, A.; Brancaleone, V.; Brogi, S.; Gojon, G.; Montanaro, R.; Morales, G.; Testai, L.; Calderone, V. Anti-inflammatory and antiviral roles of hydrogen sulfide: Rationale for considering H2S donors in COVID-19 therapy. *Br. J. Pharmacol.* **2020**, *177* (21), 4931−4941.
(67) Pacheco, A. *Sulfur-Containing Compounds as Hydrogen Sulfide Donors and Broad-Spectrum Antiviral Agents*; Ph.D. Dissertation, Washington State University: 2017.
(68) Pacheco, A.; Buchholz, D.; Monreal, I.; Xu, S.; Imbiakha, B.; Sahler, J.; Jager, M.; Lai, A.; Contreras, E.; Cook, B. New class of broad-spectrum antivirals improves influenza virus vaccine development. *Research Square* **2022**, DOI: 10.21203/rs.3.rs-1193746/v1.
(69) Diamond, G.; Molchanova, N.; Herlan, C.; Fortkort, J. A.; Lin, J. S.; Figgins, E.; Bopp, N.; Ryan, L. K.; Chung, D.; Adcock, R. S. Potent antiviral activity against HSV-1 and SARS-CoV-2 by antimicrobial peptoids. *Pharmaceuticals* **2021**, *14* (4), 304.
(70) Elazar, M.; Cheong, K. H.; Liu, P.; Greenberg, H. B.; Rice, C. M.; Glenn, J. S. Amphipathic helix-dependent localization of NS5A mediates hepatitis C virus RNA replication. *Journal of virology* **2003**, *77* (10), 6055−6061.
(71) Mariewskaya, K. A.; Tyurin, A. P.; Chistov, A. A.; Korshun, V. A.; Alferova, V. A.; Ustinov, A. V. Photosensitizing antivirals. *Molecules* **2021**, *26* (13), 3971.
(72) Dyett, B. P.; Yu, H.; Strachan, J.; Drummond, C. J.; Conn, C. E. Fusion dynamics of cubosome nanocarriers with model cell membranes. *Nat. Commun.* **2019**, *10* (1), 4492.
(73) Ferhan, A. R.; Park, S.; Park, H.; Tae, H.; Jackman, J. A.; Cho, N.-J. Lipid nanoparticle technologies for nucleic acid delivery: A nanoarchitectonics perspective. *Adv. Funct. Mater.* **2022**, *32*, 2203669.

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