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Physics of HIV

Stephanie Tristram-Nagle

Biological Physics, Physics Department, Carnegie Mellon University

Abstract

This review summarizes over a decade of investigations into how membrane-binding proteins from the HIV-1 virus interact with lipid membrane mimics various HIV and host T-cell membranes. The goal of the work was to characterize at the molecular level both the elastic and structural changes that occur due to HIV protein/membrane interactions, which could lead to new drugs to thwart the HIV virus. The main technique used to study these interactions is diffuse X-ray scattering, which yields the bending modulus, K_C , as well as structural parameters such as membrane thickness, area/lipid and position of HIV peptides (parts of HIV proteins) in the membrane. Our methods also yield information about lipid chain order or disorder caused by the peptides. This review focuses on three stages of the HIV-1 life cycle: 1) infection, 2) Tat membrane transport, and 3) budding. In the infection stage, our lab studied three different parts of HIV-1 gp41 (glycoprotein 41 fusion protein): 1) FP23, the N-terminal 23 amino acids that interact non-specifically with the T-cell host membrane to cause fusion of two membranes, and its trimer version, 2) CRAC (cholesterol recognition amino acid consensus sequence), on the MPER (membrane proximal external region) near the membrane-spanning domain, and 3) LLP2 (lentiviral lytic peptide 2) on the CTT (cytoplasmic C-terminal tail). For Tat transport, we used membrane mimics of the T-cell nuclear membrane as well as simpler models that varied charge and negative curvature. For membrane budding, we varied the myristoylation of the MA₃₁ peptide as well as the negatively charged lipid. These studies show that HIV peptides with different roles in the HIV life cycle affect differently the relevant membrane mimics. In addition, the membrane lipid composition plays an important role in the peptides' effects.

INTRODUCTION

1. Methods

The goal of this review article is to present an overview of several years' investigations from the Tristram-Nagle/Nagle lab of HIV-1 peptide/lipid interactions, studied by the method of X-ray diffuse scattering. This method obtains both elastic and structural parameters of membranes. The samples are well-oriented stacks of ~2000 bilayers on highly polished silicon wafers, hydrated through the vapor. Diffuse scattering is caused by the thermal fluctuations which occur when lipid bilayers are fully hydrated [3], which in turn causes the exiting X-ray scattering vectors to be deflected outside of the usual lamellar Bragg orders. There is actually much more information in the diffuse scatter than in the Bragg peaks, where the "lobes" of off-specular diffuse scatter yield elastic parameters as well as bilayer structure. The analysis derives from equilibrium statistical mechanics and uses a height-height pair correlation in the out-of-plane direction, where we simultaneously solve for the Caillé parameter, η , and the in-plane correlation length, ξ [3, 4]. The original Caillé

theory [7] employed a continuum model of smectic liquid crystals which has gradually been replaced by the following, more realistic, discrete free energy functional [8–12]

$$f = \frac{\pi}{NL_r^2} \int r dr \sum_{n=0}^{N-1} \left\{ K_C [\nabla_r^2 u_n(r)]^2 + B [u_{n+1}(r) - u_n(r)]^2 \right\},$$

where N is the number of bilayers in the Z (vertical) direction, L_r is the domain size in the r (horizontal) direction, K_C is the bending modulus, u_n is the vertical membrane displacement, and B is the compressibility modulus. From this analysis we are able to obtain K_C and B independently, where K_C describes the bending of a single bilayer, such as in cell membranes. While the bilayer stack is fairly thick (10 μm), it behaves as a single bilayer since a single lamellar D -spacing is measured even with inclusions, indicating that the inclusions and water have penetrated to all layers.

In 2000, Pabst et al. published the use of X-rays to study diffuse data from fluctuating lipid bilayers in capillaries, but their method relies upon isotropic samples where the elastic constants K_C and B are unable to be separated [14]. Moreover, the resolution of the electron density profiles obtained (yielding bilayer thickness) is rough, due to the use of a very simple model of the bilayer and low signal-to-noise ratio in the data. In 2001, our lab was the first to publish the X-ray diffuse scattering method to obtain both elastic moduli, K_C and B , as well as high-resolution structure from fluctuating oriented lipid membranes [3], although Salditt's lab in Germany published a similar method using specular X-ray scattering from fluctuating, oriented lipid bilayers soon thereafter in 2002 [19], and in 2006, Li's lab published an X-ray study of DOPC using oriented stacks of fully hydrated bilayers, yielding K_C and B separately [20]. More details of our low-angle X-ray scattering (LAXS) methods are given in Supplementary Material.

2. Focus on HIV-1 protein/lipid interactions

With such a powerful, probe-free method to study lipid/protein interactions, we looked for research areas where this method could yield fruitful, biologically-relevant results. Although we have published in several areas [21–27], we were attracted to the area of human immunodeficiency (HIV) due to its biological relevance and also due to its strong support at the NIH. HIV remains a health threat worldwide. Every year there are 2 million new cases, 1.5 million deaths and 37 million people living with HIV. In developed countries, the death rate from HIV/acquired immunodeficiency syndrome (AIDS) has decreased since the advent of highly active anti-retroviral therapy (HAART) in 1996. However, in the U.S., every year there are 50,000 new cases, 17,000 deaths and 1.2 million people living with HIV, so it remains a serious health problem. Therefore, any information concerning interactions of proteins, or parts of proteins, where a membrane interaction is crucial for the success of the protein, could become a future drug target. Fig. 1 summarizes the HIV life cycle, where the asterisks represent our published papers. This review article will sequentially present results obtained regarding 1) HIV viral entry into the host T-cell, 2) translocation of Tat through the nuclear membrane and 3) budding out of new HIV virions at the T-cell plasma membrane.

HIV-1 fusion protein, gp41

We begin with the HIV-1 infection step, shown in Fig. 1, which requires the fusion protein, glycoprotein41 (gp41). Fig. 2 shows a linear arrangement of well-recognized domains of gp41, where those in red type were studied by our X-ray methods. After FP23, we focus on the CRAC motif in the membrane proximal external region (MPER) of gp41, followed by the lentivirus lytic peptide2 (LLP2) on the cytoplasmic C-terminal tail (CTT) of gp41. The common thread is that all three of these domains are thought to interact with membranes during the crucial infectivity step involving fusion of the viral membrane with the target T-cell membrane.

3. FP23 in gp41

In step 1 of the 1.5 day life cycle (Fig. 1), the HIV-1 virion membrane interacts both specifically and non-specifically with the T-cell membrane. Fusion of the membrane of enveloped viruses such as HIV-1 with the target T-cell is required for infection [31]. The HIV-1 envelope glycoprotein gp160 contains two noncovalently bound subunits, gp120 and the fusion protein gp41 [32], in a trimer arrangement (“lollipops” on HIV virion in Fig. 1); the trimer is the naturally occurring form during HIV-1 infection. Numbers on gp41 in Fig. 2 indicate amino acid sequence from gp160 before cleavage into gp120 and gp41. After the initial docking step in which sites on gp120 interact with the CD4 and chemokine receptors on the target membrane [33, 34], the N-terminal hydrophobic region of the viral gp41 envelope protein is thought to provide the crucial perturbation of the target membrane that induces fusion of the viral and T-cell membranes, thus allowing the viral RNA to be injected into the host cell [35, 36]. Thus the N-terminus of gp41 (FP23, 23 amino acids) is the subject of many investigations, including three involving our lab [2, 6, 37]. Fig. 3 shows the low-angle X-ray scattering we obtained by adding FP23 to DOPC (3D) and to a thicker lipid diC22:1PC (3B) at ~16:1 lipid:peptide molar ratio. As shown, the lobes of diffuse scattering (numbered) are broader in 3B and 3D than those in Fig. 3A and 3C (controls). This increase in lateral extension was partially due to an increase in mosaic spread, or degree of misorientation, but this was not the major factor. The effect of adding FP23 to these two simple membranes was to increase fluctuations, resulting in enlarged diffuse scatter. When the decrease in white lobe intensity in the horizontal direction is analyzed in the fitting boxes shown, K_C and B are obtained using liquid crystal theory as we have described previously [3, 4]. Fig. 4 compares the fit of the liquid crystal theory to the data in Fig. 3C and 3D. Fig. 5 shows an exponential decrease in K_C with increase in FP23 in both DOPC and diC22:1PC.

As shown in Fig. 5, FP23 dramatically reduces K_C as a function of increasing concentration, lowering the energy required to bend a membrane by a factor of ~13 for the thicker diC22:1PC and ~3 for DOPC. In Fig. 6 are shown the well-known hypothetical fusion intermediates. The energy required to bend a membrane depends on K_C and also on the spontaneous membrane curvature (C_S), as $E=K_C/2(C-C_S)^2$, so lowering K_C would lower this energy. Our calculations in Ref. [2] indicate that our concentrations are physiologically relevant, and that the estimated effective radius R of the stalk can be as large as 45 Å and still attain the concentration X_e (~0.008) of FP23, where X_e is from the exponent in the exponential fit to the data in Fig. 5. However, it is possible that each FP23 might reside

primarily in one monolayer, or in a staggered arrangement in both monolayers, which could induce a permanent local spontaneous curvature in the bilayer. If so, the main result that the bending modulus decreases could be construed as FP23 inducing a local spontaneous curvature that might also reduce the energy of curved fusion intermediates. However, a staggered, regular pattern would manifest as in-plane scattering that we do not observe.

Since our methods using X-rays rely on the 1–10 nm scale, we attempted to verify if the more traditional methods of obtaining K_C , such as micromechanical manipulation (pipette aspiration) (MM) and shape or fluctuation analysis (SA), which both use giant unilamellar vesicles (GUVs) at the micron length scale, would confirm our dramatic results for FP23. We resolve this issue in the next paragraph, but first here is some background. For pure lipids, the absolute values of K_C from our X-ray methods are in good agreement with MM [38], while SA results give larger K_C values by a factor of 1.5–2 [39]. To try to understand this difference between X-ray and SA, we tested whether the sugars that are required for both these methods alter K_C , but we found no effect of glucose or sucrose [40], so that cannot be the reason for the disparity. Recently, the Nagle lab has included a new elastic modulus, molecular tilt, which was initially shown to increase the absolute value of K_C by ~20% [41]. However, newer data with many pure lipids [42] or as a function of temperature [43] yield a K_C with tilt analysis that is ~1.5 times higher than without considering tilt, thus removing the disparity between X-ray and some SA studies. All of the measurements discussed in this review were carried out without the newer molecular tilt field analysis since we are not so concerned with absolute values of K_C , rather with the relative effect of peptides on the pure lipid K_C 's. In general, the effect of HIV peptides on K_C is larger than a factor two.

In order to compare our FP23 results with SA and MM, Dr. Tristram-Nagle teamed with Dr. Rumiana Dimova's lab where postdoc Pavlo Shchelokovskyy carried out data collection and analysis. Fig. 7 shows the effect of FP23 in DOPC on bending modulus (rigidity) K_C (κ) using MM, SA and X-ray scattering, normalized to 1 to avoid differences in absolute values. As shown, there was a general decrease in K_C due to added FP23 in all cases, but slightly less for MM and SA than for X-ray. The small discrepancy of MM and SA compared to X-ray could be due to the different length scales in these methods. This decrease in the bending rigidity is similar to that observed with co-surfactants [44] and is theoretically expected for transmembrane inclusions [45–47].

In a separate collaboration with the Weliky and Kooijman labs, we investigated spontaneous curvature as well as K_C when FP23 was added to a more heterogeneous membrane mimic [6]. LM3 is a T-cell plasma membrane mimic consisting of a mixture of six lipids: POPC:POPE:POPS:SoyPI:Egg SM:chol in a 10:5:2:1:2:10 mole ratio. For this study, we were fortunate to obtain the trimer version of FP23 due to the efforts of Wei Chang, a graduate student at Michigan State. As shown in Table 1, we found that K_C was also dramatically decreased in LM3 due to the effects of FP23 and of FP-trimer (FPtri). We also tested a water-soluble monomeric form of FP23, FPwsm. Even though this peptide was initially co-solubilized in organic solvent with the lipid, it partitioned into the aqueous phase and had little effect on LM3. When electron density profiles were constructed, we found that both FP23 and FPtri localized near the interfacial region, as shown in Fig. 8 (dotted

lines). At this location, FP23 can act as a wedge to induce curvature in a flat bilayer, thus facilitating the formation of fusion intermediates.

While the work reviewed so far used diffuse scattering, a related standard method to study spontaneous curvature employs X-ray scattering from lipids such as DOPE that form hexagonal II phases [48, 49]. Using this method it is possible to quantify curvature as the inverse of the radius of the pivotal plane, defined as that radius at which the area does not change as the monolayer bends upon hydration. Fig. 9 shows the location of the pivotal plane (R_{OP}) as well as other locations of interest, which include R_W , the radius of the H_{II} water cylinder. In addition, R_S is the radius from the center of the H_{II} tube to the lipid steric thickness, which bisects the headgroup region with associated water; R_C is the radius to the start of the hydrocarbon region; R_1 is the effective lipid radius, including void region. Using these radii and the equations of Leikin et al. [50], we calculate three areas: A_W , the area per lipid molecule at the Luzzati plane (R_W), A_P , the area per unit cell at the pivotal plane (R_{OP}), and A_1 , the area corresponding to R_1 . Table 2 gives numerical values for these quantities. The main result is that FP23 decreases R_{OP} , thereby increasing the spontaneous curvature of the mixture beyond that of the already highly curved H_{II} DOPE lipid. Since $1/R_{OP} =$ the spontaneous curvature (C_S), then the energy for forming fusion intermediates is further reduced beyond the K_C reduction, since $E=K_C/2(C-C_S)^2$.

5. CRAC motif on MPER in gp41

The membrane proximal external region (MPER) region is in close proximity to the transmembrane region of the HIV-1 membrane and this region contains a highly-conserved consensus sequence having the pattern L/V-X(1–5)-Y-X(1–5)-R/K in which X(1–5) represents 1–5 residues of any amino acid. In HIV-1 this sequence is LWYIK, which has been shown to bind to cholesterol; mutations in the CRAC sequence reduce HIV infectivity [51]. In a collaboration with the Epanand lab [5], LWYIK was compared to IWYIK, a non-CRAC motif peptide, in the absence and presence of cholesterol. Previous calorimetric work using SOPC/cholesterol (1:1 molar ratio) mixtures suggested that LWYIK binds directly to cholesterol, while IWYIK binds to SOPC [52, 53]. In Ref. [5] in addition to obtaining K_C , we obtained S_{xray} , a chain order parameter. This method was developed in our lab due to the efforts of Thalia Mills, our postdoc [54, 55]; it analyzes diffuse X-ray scattering in the wide-angle region due to chain-chain correlations. The analysis uses a Mauer-Saupe distribution of tilt angles to model the chain disorder to yield S_{xray} , which was shown to parallel S_{CD} from NMR [54]. Table 3 shows the K_C and S_{xray} results when LWYIK and IWYIK were added to SOPC or SOPC/cholesterol (30 mole percent). As shown, both LWYIK and IWYIK at 9:1 lipid:peptide mole ratio decreased K_C , thus softening SOPC or SOPC/cholesterol, where this effect was slightly greater with IWYIK. In addition, both peptides disordered lipid chains as evidenced by a decrease in S_{xray} . More details of our wide-angle X-ray scattering (WAXS) methods are given in Supplementary Material. Table 3 also shows structural results that are obtained using form factors and electron density profiles as described below.

In order to obtain electron density profiles, form factor data are collected from the intensity patterns, similar to those shown in Fig. 3, as described previously [3, 4]. Form factor data are

shown in Fig. 10 for SOPC containing LWYIK (L) or IWYIK (I). Fig. 10 shows a shift of the form factor data of SOPC containing either LWYIK or IWYIK to higher q_z , indicating a thinning of the SOPC bilayer. When the form factor data are fit to a model of the bilayer, electron density profiles are constructed [57] (shown in Fig. 11), and structural changes are quantitated. Thinning of the hydrocarbon thickness ($2D_C$) by ~ 3 Å was caused by the addition of either LWYIK or IWYIK, and both peptides located in the headgroup region near the phosphate group in pure SOPC. When 30% cholesterol was added to SOPC, then both peptides located in the hydrocarbon region, ~ 10 Å from the bilayer center (previously unpublished). Therefore, these structural, elastic and chain order results surprisingly did not exhibit large differences between LWYIK and IWYIK.

However, when the concentration of cholesterol was increased to 50 mole percent, which is closer to the 45 mole percent in the HIV viral membrane [58], dramatic differences were observed in the ability of LWYIK vs. IWYIK to crystallize out cholesterol. Either peptide induced the initial formation of oriented, 3D crystals during the rock and roll thin film procedure [37], which produced a highly crystalline oriented pattern as shown in Fig. 12A and B, as compared with a few unoriented crystalline reflections in the wide-angle pattern of dried SOPC/50% cholesterol membranes (12C). The crystalline pattern in Fig. 12A caused by IWYIK had stronger, sharper reflections in several samples than did the LWYIK patterns, where in both patterns the spacings are consistent with the ~ 17 Å dimension of the cholesterol molecule [5]. When these samples were hydrated, the crystalline patterns dissipated, but more rapidly for the crystals formed by LWYIK. We interpret these patterns to indicate that LWYIK binds directly to cholesterol, thus interfering with its ability to form highly crystalline arrays, while IWYIK binds to the nearby SOPC lipid, thus sequestering cholesterol away from the lipid, allowing it to form long-range, highly crystalline arrays. This could be an indication that in vivo, LWYIK binds directly to cholesterol and aids in the fusion process of the viral and host cell membranes.

Our result that LWYIK resides in the hydrocarbon interior in SOPC/Chol (7:3) membranes, but in the headgroup region in SOPC membranes, disagrees with the result of the Brown lab [59], who found the CRAC motif bacterial leukotoxin to remain at the bilayer surface of ULV containing 33% cholesterol using fluorescence and MD simulation [60], and also disagrees with the molecular simulation of Epanand's lab that found LWYIK to reside at the interface in cholesterol-containing membranes [61]. However, in another investigation from the Epanand lab, a nearest neighbor recognition (NNR) study demonstrated that LWYIK is sensitive to the packing of the bilayer, suggesting that the peptide must at least partially penetrate into the membrane [52]. The fact that LWYIK caused the S_{xray} value to decrease by a factor of 2 in SOPC/Chol (7:3) membranes, while only slightly in SOPC membranes, supports the differential location of the CRAC motif found in the electron density profiles in our study. In another study, fluorescence measurements of the tryptophan moiety showed that either LWYIK or KIYWL tethered to cholesterol or to a phospholipid favor the bilayer interface region in liquid-disordered lipids [62]. This agrees with our location of free LWYIK in pure SOPC membranes.

6. LLP2 on CTT in gp41

The motivation for this study was to determine how a moiety on the cytoplasmic C-terminal tail (CTT) of the HIV-1 virion (see Fig. 2) is able to affect the fusion process that occurs externally. The biochemical question is: How does a signal get transmitted to the other side of the membrane? The physics question is: How does the membrane carry information from one side to the other? Three conserved amphipathic, primarily α -helical segments in the CTT referred to as lentiviral lytic peptides (LLP1, LLP2 and LLP3) have been shown to bind and perturb membranes [63–67]. To understand the function of the CTT, deletions of the entire CTT have been carried out with different results depending upon cell type. For so-called permissive cell types, deletion has little effect on Env (gp120 and gp41) incorporation into virions [68, 69]. However, the majority of T-cell lines are non-permissive in that CTT deletion results in a 10-fold reduction in Env incorporation and a complete blockage of viral replication [70]. Murakami and Freed found the block to be at the early stage of Env incorporation [68]. Less drastic changes in the CTT have been carried out using site-directed mutations. Kalia et al. [71] produced a mutant MX2 of LLP2 where two highly conserved arginine residues were replaced with negatively charged glutamic acid, thus decreasing the net charge from +3 to -1. In HIV virions, MX2 mutation showed no loss of Env expression or infectivity, while when MX2 was in the T-cell membrane, both viral-initiated cell death and syncytium formation (cell-cell fusion) were greatly decreased [71], which was similar in permissive and non-permissive cell types. In Ref. [15] we collaborated with the Montelaro lab to employ membrane mimics of both HIV-1 virion and T-cell membranes, and added WT LLP2 and the MX2 mutated form of LLP2. The lipid compositions were based on [58, 72] and are shown in Table 4. In addition to the HIV, HIV minus negative charge and T-cell membrane mimics, an HIV mimic devoid of cholesterol was also attempted, but this membrane phase separated into fluid and gel phases and was thus not analyzable. A lipid extract from attenuated HIV-1 virions provided another membrane mimic.

When the diffuse X-ray scattering data were analyzed as detailed above, the results in Table 5 were obtained. Both WT LLP2 and MX2 caused membrane softening as indicated by a lowered K_C , which was even lower for MX2, in T-cell membrane mimics. There was little change in S_{xray} order parameter, indicating that the lipid chains were not the cause of the membrane softening. In the HIV and HIV-neg mimics, the K_C value was quite low for the control lipid, indicating a soft membrane even before peptides were added. This was also true for the membranes created from lipids extracted from HIV virions. These lower K_C values (6–8.2/kT) are surprising because of the high cholesterol content (45 mole %) of these membranes [56]. The K_C values and S_{xray} order parameters were little affected by the addition of either peptide to HIV, HIV-neg or HIV extracted membranes. Therefore, this study showed that very realistic mimics of the T-cell and HIV-1 virion membranes have quite different elastic moduli. Since both WT and MX2 lowered K_C , as we saw previously with FP23 and with the CRAC motif, this cannot be the source of the difference between their mechanisms of action. To explore this further, we obtained electron density profiles.

Fig.13A shows a typical form factor and fit to the Scattering Density Profile (SDP) model program [73] from the T-cell X-ray data with WT, 40:1 lipid:peptide molar ratio [15]. When X-ray data from HIV membrane mimics were analyzed, there was no difference in the form

factors when peptides were added compared to the control, indicating that the peptides had not penetrated the HIV membranes. This is the reason that there was also no change in K_C or S_{xray} . Fig. 13B shows the electron density profiles for T-cell membranes and with 40:1 lipid:peptide mole ratio. As shown, there was slight thinning in membrane thickness when WT LLP2 was added, while there was a greater thinning when MX2 was added to the T-cell mimic. In addition, there was a deeper penetration of MX2 into the hydrocarbon core, while WT LLP2 remained outside of the phosphate headgroup. A theoretical challenge is to understand how the cytoplasmic CTT influences the extracellular domain of the gp41 Env protein. A potential biophysical mechanism involves the lateral pressure profile of the membrane [74], which would be affected locally by embedded LLP2 and which would then affect other nearby parts of gp41, such as the membrane spanning domain (MSD). Perturbation of the MSD of gp41 could affect the conformational changes necessary for fusion as several studies with modified MSDs have demonstrated inhibition of fusion [75–77]. With regard to the T-cell membrane, the deeper embedding of MX2 would alter the lateral pressure profile compared to WT and that would affect the TMD of the gp41 Env protein differently, correlating well to the inhibition of cell-cell fusion upon mutation. With regard to the HIV membrane mimic, the very weak interaction of LLP2, either WT or MX2, predicts that MX2 would have little effect on Env incorporation or infectivity, as observed experimentally. The cartoon in Fig. 14 summarizes the interaction of LLP2 WT and MX2 with the two membrane mimics.

7. Tat_{47–57} peptide

As shown in Fig. 1, Tat translocates through the T-cell nuclear membrane to enhance transcription of DNA into RNA. Two investigations from our lab [16, 17] focused on this transactivator of transcription, Tat, which plays a role in AIDS progression. Tat peptide Y₄₇GRKKRRQRRR₅₇ is part of a larger 86-amino acid protein that transactivates a gene on the HIV-1 promoter [78, 79], and is thought to be responsible for cellular uptake [80]. In order to do this, Tat must translocate from the cytoplasm into the nucleus across the nuclear membrane. Tat peptide_{47–57} is particularly rich in basic amino acids; deletion of three out of eight positive charges in this region caused loss of its ability to translocate [80]. Many cell penetrating peptides (CPPs), including those derived from Tat, are capable of carrying cargo into live cells [81, 82]. It is controversial, however, if endocytosis is involved, which would require ATP [80, 83–86]. To address this question, many biophysical studies have used simplified models of biological membranes composed of a small number of lipid types including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylglycerol (PG). For example, Mishra *et al.* reported that rhodamine-tagged Tat_{47–57} enters giant unilamellar vesicles (GUVs) composed of PS/PC (1:4 mole ratio) immeasurably slowly, but crosses a GUV composed of PS/PC/PE (1:2:1) lipids within 30 s [87]. In another experiment, fluorescently labeled Tat_{48–57} did not enter GUVs containing only PC with 20 mole % cholesterol, but translocated rapidly when PS or PE was included with PC [88]. Therefore, Tat-derived peptides can translocate across bilayers without ATP, but this depends on the lipid type. The mechanism by which bilayer translocation occurs remains unknown; our study attempted to delineate Tat's effect upon and location in membrane mimics in order to elucidate the mechanism of Tat's translocation.

In both of our investigations we collaborated with Angel Garcia's group, pairing MD simulation with our X-ray data. Grad student Kiyoo Akabori led the experimental research effort in our lab. Using the Sim-to-Exp program [89], form factor data are calculated from the simulation's trajectories; these are compared directly to the form factors that result from the X-ray data. Since peptides move slowly in atomistic simulations, grad student Kun Huang in the Garcia lab constrained the peptide positions using umbrella potentials at desired depths with a force constant of 3000 KJ/mol/nm². Figure 15 shows one of these comparisons. In Fig. 15A, a mixture of Tat and the neutral lipid DOPC (33:1 lipid:peptide mole ratio) was simulated (red line) and compared to the X-ray form factor data (open black circles) of the same mixture. As shown, there is good visual agreement when Tat is constrained to 18 Å from the bilayer center (in the headgroup region). A snapshot of this simulation is shown in Fig. 15B. By contrast, when Tat was constrained to 5 Å from the bilayer center, i.e., near the center of the bilayer, the fit to the data was much worse and the chi-square was larger (see legend). Therefore, these results suggested that Tat does not translocate easily through DOPC. Fig. 16 shows the electron density profile corresponding to Fig. 15A. These comparisons informed construction of the cartoon shown in Fig. 17.

In the same investigation, four other membrane mimics were also studied [16] (see Fig. 18). In all of these mixtures, Tat peptide decreased the bending modulus K_C as shown in Fig. 18. Thus, like FP23, Tat lowers the energy required to bend a membrane which could be crucial for its translocation through membranes. While Ref. [16] showed a clear preference for the headgroup location for Tat in DOPC, two locations, in the headgroup, and near the bilayer center, were found for the DOPC:DOPE 1:1 mixture as detailed in the Supplementary Material to Ref. [16]. To pursue this further, the second investigation [17] focused on the DOPC:DOPE 1:1 lipid mixture. Due to the efforts of Garcia's postdoc, Chris Neale, a matrix of Tat positions was investigated, also varying the area/lipid A_L and the Tat concentration. In total Chris carried out over 500 100-nsec atomistic GROMACS simulations. As shown in Fig. 19A, at the lower concentration of Tat in the lipid mixture, two clusters of blue squares, indicating a low chi-square, appear. One cluster is near $z_{\text{Tat}} = 16\text{--}18$ Å and a second cluster is near $z_{\text{Tat}} = 0\text{--}8$ Å. The smallest chi-squares are shown on the form factor graphs in 19B and D, where the solid lines represent the simulated form factors and the open black circles the experimental form factors. This indicates that it is equally probable to find Tat near the center of the bilayer as in the headgroup region for a lipid:peptide molar ratio of 67:1. At the higher concentration, 33:1, Tat remains in the headgroup region, possibly due to crowding. When Tat is constrained at 8 Å from the bilayer center, if bilayer undulations are large, then Tat could be located in the headgroup region. This is shown in Fig. 20C and D. However, in those cases, the chi-square of the fit increases dramatically, thus ruling out these possibilities. What is remarkable is that in the case of the favored position in Fig 20B, Tat spans the bilayer, interacting with the headgroups of both bilayer leaflets and drawing water and ions into the bilayer's hydrophobic core. When Tat is restrained at 8 Å from the DOPC:DOPE (1:1) bilayer center, the hydrocarbon thickness $2D_C$ decreases by 2.5 Å, which leads to a 6 Å² increase in A_L when volume is also measured.

Our main results that Tat₄₇₋₅₇ in DOPC remains in the headgroup region, and that it is located either in the headgroup region or near the center of the bilayer in DOPC:DOPE (1:1) mixtures, supports a previous suggestion that non-bilayer forming lipids are required

for Tat translocation across membranes (Ref. [91] and references within). To compare to literature, Bradshaw's lab has published two neutron studies of Tat in membranes. Ref. [92] studied mixtures of Tat with DOPC:DOPS (80:20 molar ratio) and found Tat to be located in the headgroup near the glycerol/carbonyl region, similar to our result for Tat in pure DOPC [16]. However, in pure DOPC, Bradshaw's lab found Tat to be located deep in the hydrocarbon region, ~ 6 Å from the bilayer center [93]. This is in direct contradiction to our result, where we found that DOPE is necessary for Tat internalization in the hydrocarbon core. The reason for these different results could stem from the degree of hydration. In their experiment, the highest hydration level was 98% relative humidity (RH). We have previously shown that even at 99% RH, oriented bilayers are still 10 Å (~ 10 water molecules) below their full hydration value [23]. When there is not enough water between bilayers in a stack, then a peptide may be forced into the hydrocarbon region. In our experiment, there is more than sufficient water for the peptide to partition naturally. In another study, Shin's lab used neutron reflectivity (NR) of Tat in a DPPC bilayer to show that Tat entered into the hydrocarbon region [94]. However, high temperature and high concentration manipulation were required for this result, since their NR measurements occurred at 25 °C, where DPPC is in the gel phase, so their result may not be physiologically relevant.

8. HIV-1 matrix-31 membrane binding peptide

Retroviral replication occurs as the virus acquires its lipid envelope at the infected cell's plasma membrane (PM) [95], as shown in Fig. 1. Many complex steps occur during viral budding, which is mediated by the viral structural protein Gag [96]. Human immunodeficiency virus (HIV-1) Gag is synthesized as a precursor polyprotein, Pr55Gag, which is cleaved into four major domains upon release: N-terminal matrix (MA), capsid (CA), nucleocapsid (NC) and P6 [97]. The membrane binding domain is MA, which was the subject of our study [18]. This binding depends on a conserved highly basic region (HBR) in the 131 amino acid MA protein [98, 99], as well as on the myristoyl moiety at MA's N-terminus. The binding is enhanced when a conformational switch removes the myristate moiety from its sequestered domain [100], as the HBR interacts with acidic phospholipids on the inner leaflet of the PM [98, 100]. Gag is targeted to the PM due to the interaction between the MA HBR and phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂ or PIP₂), where it is concentrated. Specific binding of HIV-1 Gag to PIP₂ has been demonstrated by several experimental approaches [72, 101, 102]. In our work, interactions between the HIV-1 Gag MA membrane binding interface with lipid bilayers were studied using X-ray diffuse scattering to obtain the membrane bending rigidity, chain order, area/lipid and bilayer thickness. In a collaboration with the Lösche/Heinrich lab, we used NR to determine the peptide position in bilayers. The model peptide, called MA₃₁myr, includes the main membrane interaction sites, myristoyl and the HBR, of the full-length MA protein. For contrast we also studied MA₃₁ without the myristoyl group. We compared the interaction of MA₃₁ and MA₃₁myr with PIP₂ or PS-containing membranes in an effort to understand the requirement of PIP₂ for viral budding.

Similarly to the above studies, we first determined K_C , the bending modulus of different lipid/peptide interactions. As shown in Fig. 21A, there was a greater decrease in K_C when MA₃₁ was added to the neutral lipid POPC than when MA₃₁myr was added (black squares).

This was at first surprising, but it indicates that the myristoyl group has a stabilizing effect on a neutral membrane. However, when 20 mole % of PIP₂ was added to POPC, the difference caused by the myristoyl group disappeared, indicating that the electrostatic interaction dominated. The negative charge on PIP₂ caused a decrease in K_C in the presence of either peptide. Without the peptides, adding PIP₂ to POPC caused only a small decrease in K_C (21C, black squares). Fig. 21B again shows that differences between MA₃₁myr and MA₃₁ were largely suppressed by the addition of any of the negatively charged lipids, PI, PIP or PIP₂ to POPC/POPE mixtures. In Fig. 21C, all differences between MA₃₁myr and MA₃₁ are gone when the overall net negative charge is at least -0.26 . Fig. 21D shows a dramatic difference between the behavior of PS-containing membranes and PIP₂-containing membranes: those with PS first stiffen the membrane as either peptide is added, while those with PIP₂ are generally softened (lowered K_C), with either peptide. These lipid mixtures were designed to achieve equal overall net negative charge.

Fig. 22 shows the chain order S_{xray} results as a function of peptide concentration for the series of samples in Fig. 21D. Chain order initially increased, then decreased with increasing concentration of both peptides in POPC:POPS (60:40) membranes, similar to the trend observed in the K_C results. The structural results in Fig. 23 followed a similar trend; first a membrane thickening followed by a membrane thinning at higher peptide concentration. The thickening is due to an increase in chain order which causes the lipid chains to extend, but at higher concentrations this reverses, causing the membrane to thin back to the control value. In contrast to POPC/POPS, a very gradual decrease in membrane order occurred in the POPC/PIP₂ (90:10) samples (Fig. 22), which is consistent with the 2.5 Å membrane thinning that occurred (Fig. 23D). Concomitant with membrane thinning, there was an increase in A_L with added peptide(s) in the POPC/PIP₂ samples.

In this work, we used both X-ray diffuse scattering and neutron reflectivity to determine the location of both peptides in membrane mimics. Neutron reflectivity is superior for obtaining peptide position in a bilayer due to a greater contrast between the protein, lipid headgroup and lipid hydrocarbon chains [103], while X-ray scattering is well suited to reporting changes in membrane thickness and elastic properties. NR results (Fig. 24) show MA₃₁myr located both in the headgroup (HG) region as well as $69 \pm 17\%$ in the hydrocarbon (HC) region in both membrane mimics. There were no significant differences between MA₃₁ and MA₃₁myr, within the 68% confidence limits. The striking result is that the MA peptides reside quite deeply in the interior of charged membrane mimics, deeper than predicted from hydrophobicity scales [104]. This can be understood in terms of the cartoon in Fig. 25, which shows a proposed distribution of the peptides along the membrane normal. If the charged amino acid side chains are self-neutralized, this can allow greater hydrophobic penetration. As arginines and lysines are neutralized, their side chains become hydrophobic moieties.

Our main conclusions are that substitution of PIP₂ by PS lipids of equivalent charge changed the effect of MA₃₁'s binding on many membrane properties. In addition to the recognized function of PIP₂ of providing a stereo-specific anchor for the MA protein to the membrane [101], reducing the bending modulus reduces the free energy for restructuring the membrane for viral budding. Our other results show that only when a strong charge neutralization does

not dominate the interaction does the myristoyl group aid MA₃₁ binding. Overall, these results emphasize the importance of the lipid composition in MA₃₁ peptide binding.

9. Concluding Remarks

This review article has summarized the HIV-1 work published in the Tristram-Nagle/Nagle lab during over a decade of funding from the National Institutes of Health. The results show particularly that elastic properties of membranes are sensitive to the effects of the membrane-binding portions of proteins from the HIV-1 virus. How the bending modulus of the control membrane mimics responds to the peptides depends strongly on the lipid composition, as well as on charge, length and hydrophobicity of the peptides. In addition, chain order is a second sensitive measure of interactions. Thus, physics has provided a unique window using probe-free scattering techniques into detailed information concerning peptide/lipid interactions. Structural information is also obtained from the X-ray and neutron scattering, regarding bilayer thickness and depth of insertion of parts of proteins into the relevant membranes. When X-ray data are combined with MD simulation, molecular details of the interactions are observed and the simulation is validated. Information provided by these studies may be used to guide the design of new drugs to thwart the HIV virus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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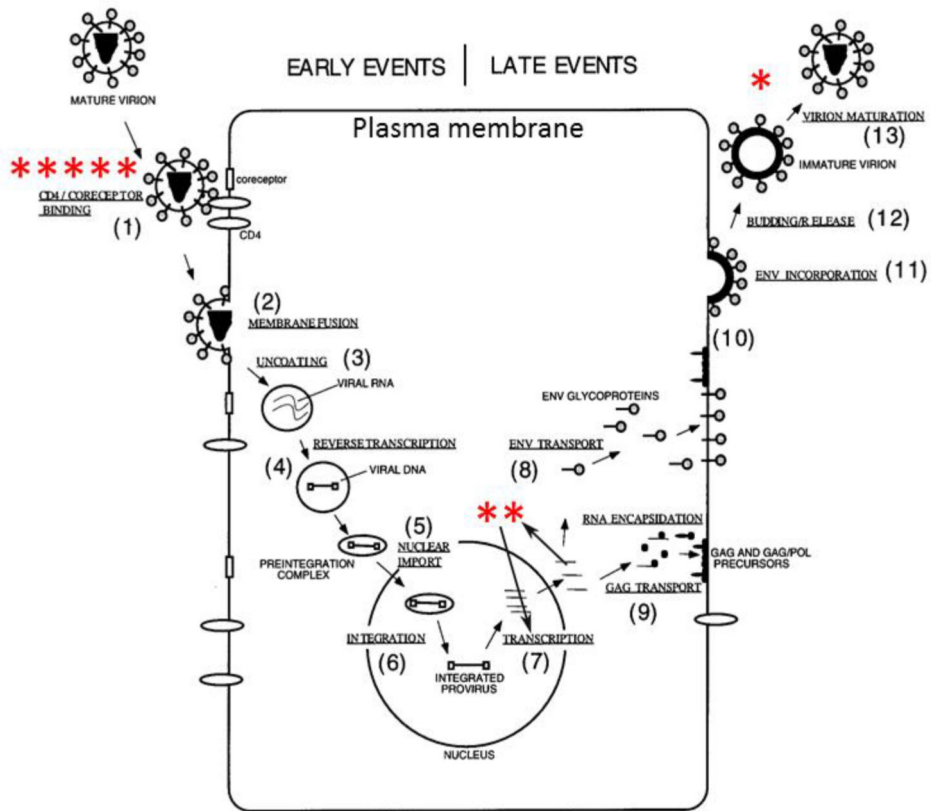


Figure 1. HIV life cycle (figure adapted from Ref. [1]). Asterisks represent works published by the Tristram-Nagle/Nagle lab at 3 steps in the HIV life cycle: HIV entry *****[2], [5], [6], [13], [15], Tat translocation **[16], [17], and budding of new HIV virions *[18].

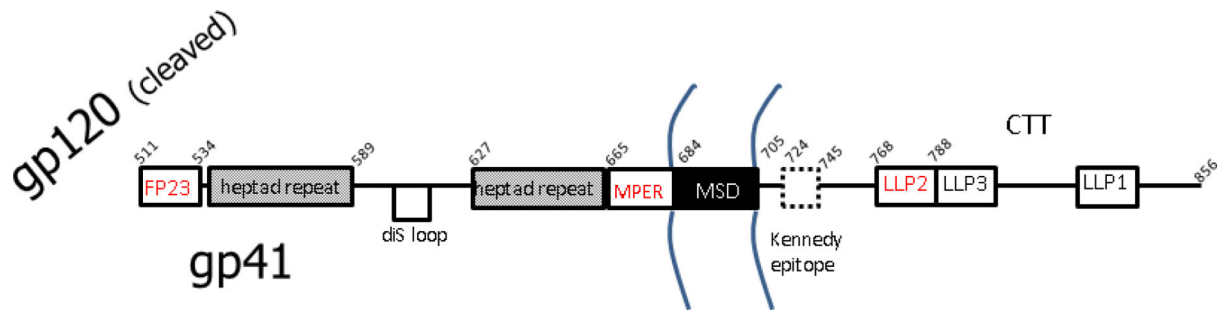


Figure 2.

Linear sequence of recognized epitopes in gp41, adapted from Refs. [28–30]. Abbreviations: disulfide loop (diS loop), membrane proximal external region (MPER), membrane spanning domain (MSD), lentivirus lytic peptide (LLP). The region to the right of TM is the cytoplasmic C-terminal tail (CTT) [30]. Dotted line for the Kennedy epitope indicates that it sometimes is exposed extracellularly [30]. Curved lines indicate the membrane.

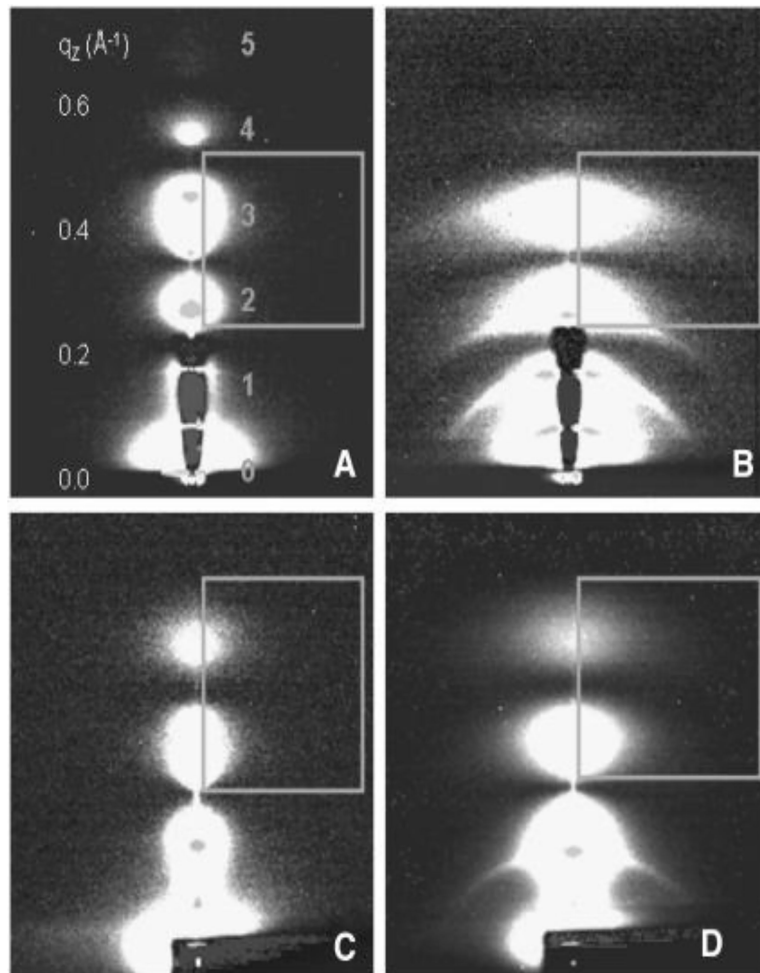


Figure 3. 2D CCD scattering intensity images obtained at CHESS at 30 °C of (A) diC22:1PC, (B) FP23/diC22:1PC (1:15) (C) DOPC, and (D) FP23/DOPC (1:17). The white regions define diffuse scattering lobes that are numbered 1–5 in A. The lamellar Bragg orders appear as narrow, horizontal lines, showing through the semi-transparent beam-stop in A and B, or as dots on the lobes in all images. The gray boxes are the fitting boxes within which the diffuse scattering theory is used to fit to the data to obtain K_C and B . The samples are oriented stacks of ~2000 bilayers on highly polished silicon wafers prepared by evaporation of organic solvent using the rock and roll method [37]. The dried lipid/peptide films are hydrated through the vapor in a thick-walled hydration chamber [24] to full hydration. The flat samples are rotated between -3° and 7° during 30–60 second dezingered exposures. Figure from Ref. [2].

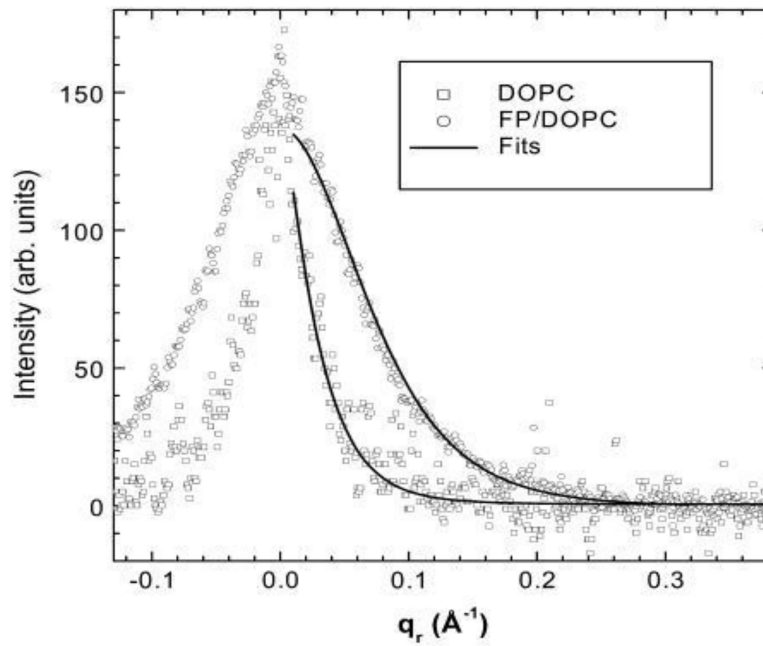


Figure 4. **(left).** Normalized and background subtracted DOPC and FP23/DOPC (1:17) diffuse scattering data as a function of q_r at the position of the highly diffuse 5th order peak are shown by open symbols. The corresponding fits from the diffuse scattering theory are shown by black lines. Figure from Ref. [2].

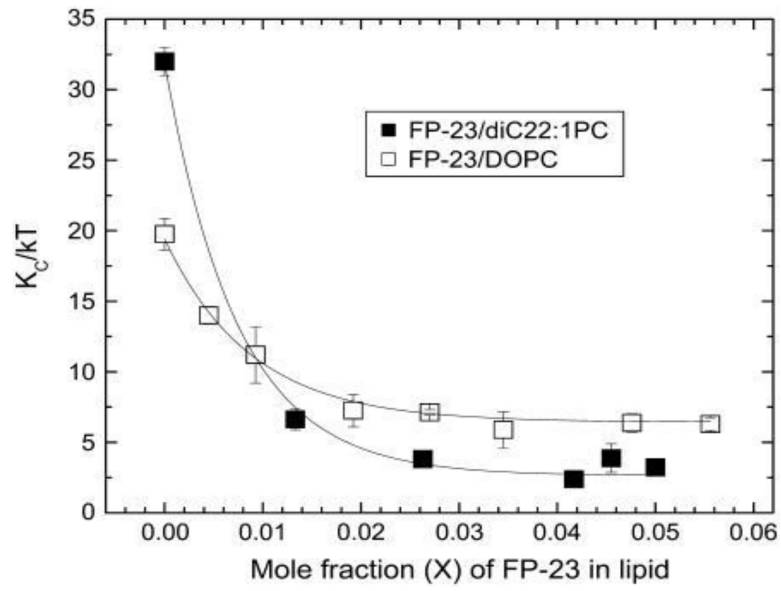


Figure 5. (right). Effect on bending modulus K_C , in units of thermal energy kT , when adding FP23 to diC22:1PC and to DOPC. The lines are exponential fits to the data. Figure from Ref. [2].

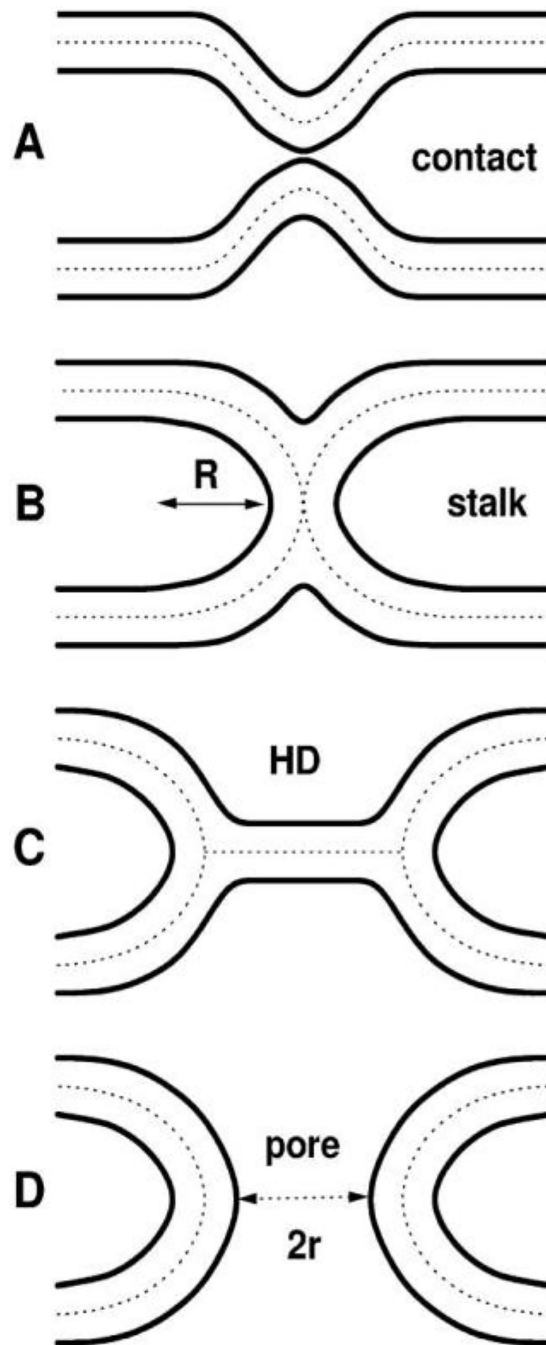


Figure 6. Well-known hypothetical fusion intermediates. Lipid bilayer surfaces are indicated by solid lines. Dotted lines in the hydrocarbon interior divide the bilayers into monolayers. A. Contact of the virus and target membranes, B. stalk that allows lipid mixing, C. hemifusion diaphragm (HD), D. pore. Figure from Ref. [2].

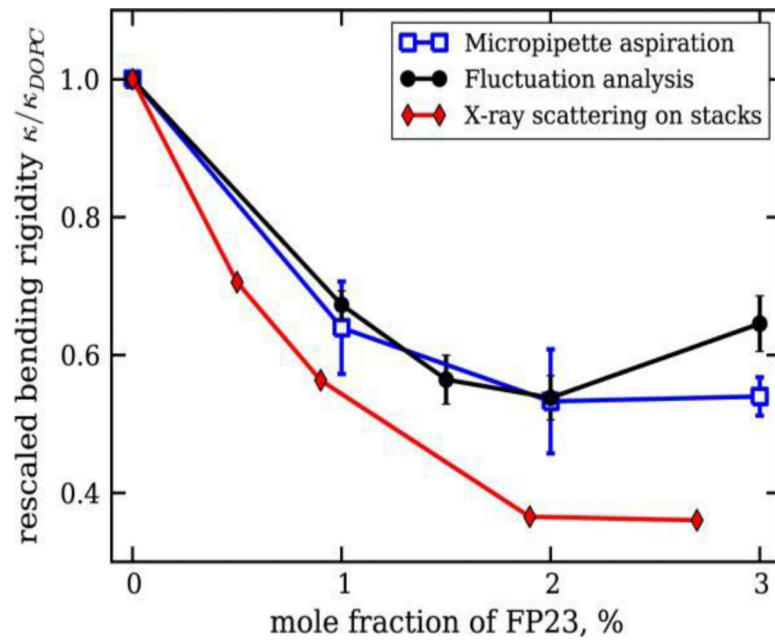


Figure 7. Relative changes of the bending rigidity as a function of the FP23 concentration in DOPC bilayers. See legend for details. Figure from Ref. [37].

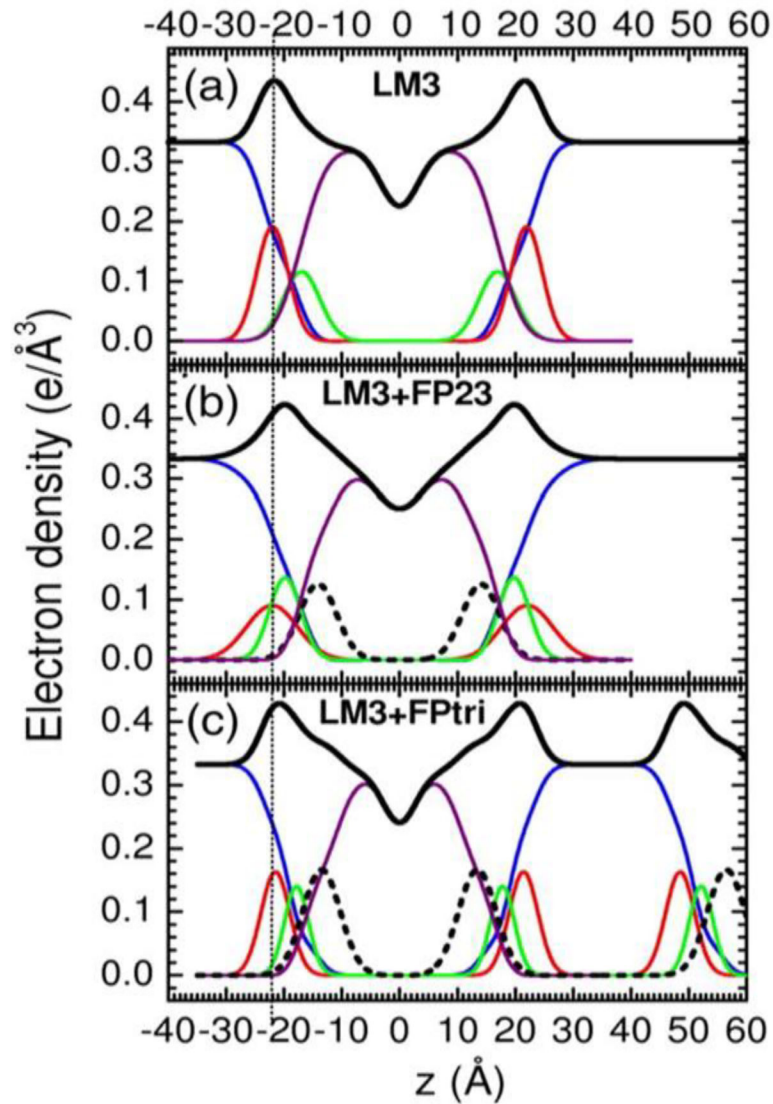


Figure 8. (right). Electron density vs. distance (z) from the bilayer center for samples of (a) LM3, (b) .048 mole fraction FP23/LM3, and (c) .016 mole fraction FPtri/LM3. Line colors: Black is total electron density, blue is water, red is phosphate plus average headgroup moiety, green is glycerol-carbonyl, purple is hydrocarbon region including cholesterol, black dotted line is peptide. (c) shows part of a second adjacent bilayer at large values of z , and the presence of a large water spacing between adjacent bilayer is emphasized in all panels. Adapted from Ref. [6].

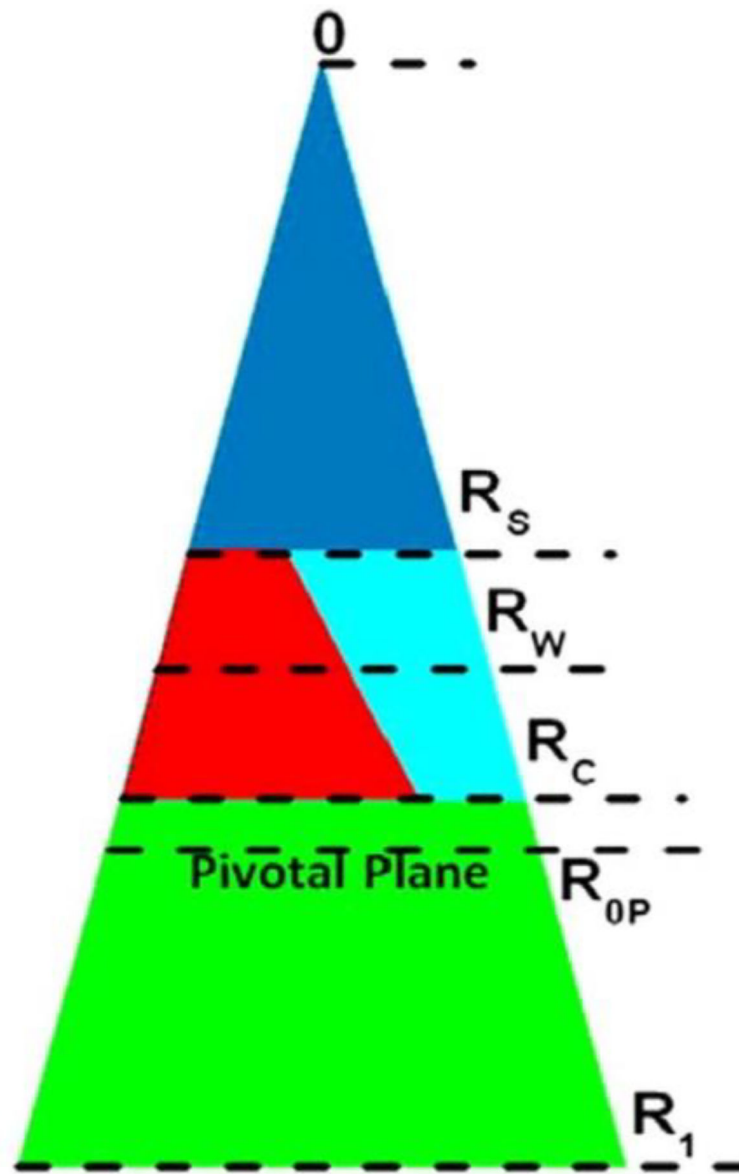


Figure 9.

Schematic for the packing of lipid and water in the hexagonal II phase. The cylindrical water tubes extend in the direction perpendicular to the page, with the tube's center at 0. The triangle extending from 0 to R_1 represents the piece-of-pie space occupied by a monolayer of lipid and peptide and its associated water. The dark blue triangle is occupied by water. The lower green trapezoid is the hydrophobic volume. The region between R_S and R_C represents the interfacial region, which is occupied by water (light blue) and lipid headgroup (red). FP23 is located partially in the red region and partially in the green region. The pivotal plane is at R_{0P} . Figure from Ref. [6].

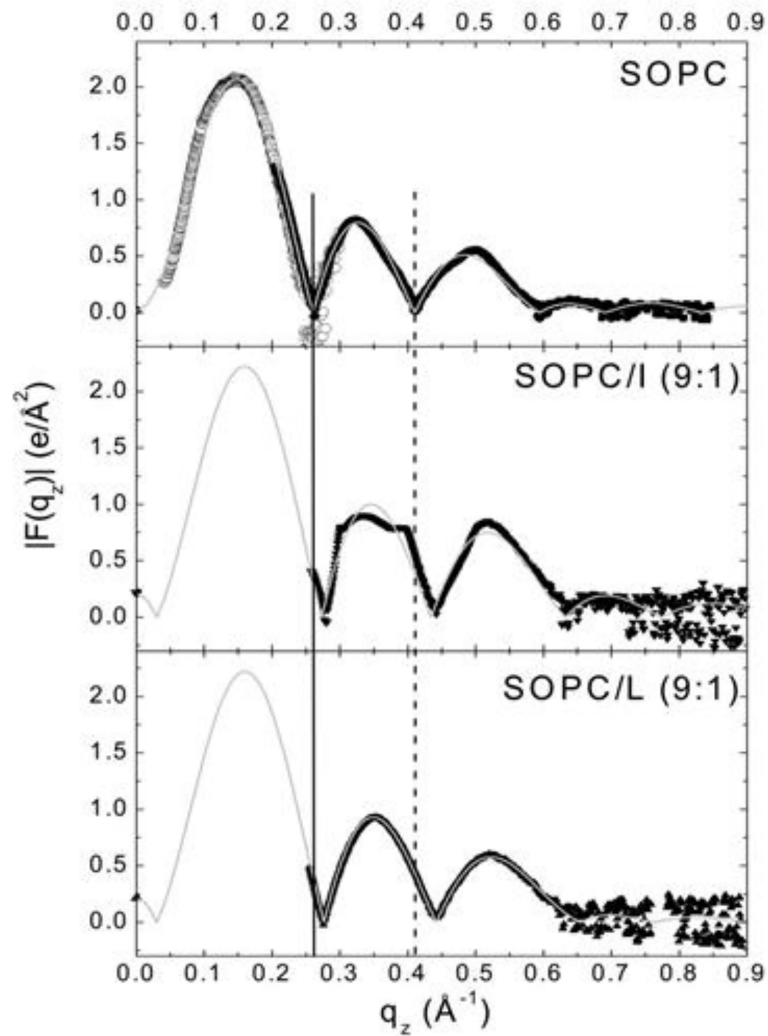


Figure 10.

(left). $|F(q_z)|$ data for SOPC, SOPC/IWYIK (9:1) and SOPC/LWYIK (9:1). Solid symbols represent data from oriented samples. Open symbols for only SOPC were obtained from unilamellar vesicles (ULV). The solid and dotted vertical lines show shifts in the q_z values for the zeros compared to SOPC. The thin solid curves show the fits of the model to the data. Figure from Ref. [5].

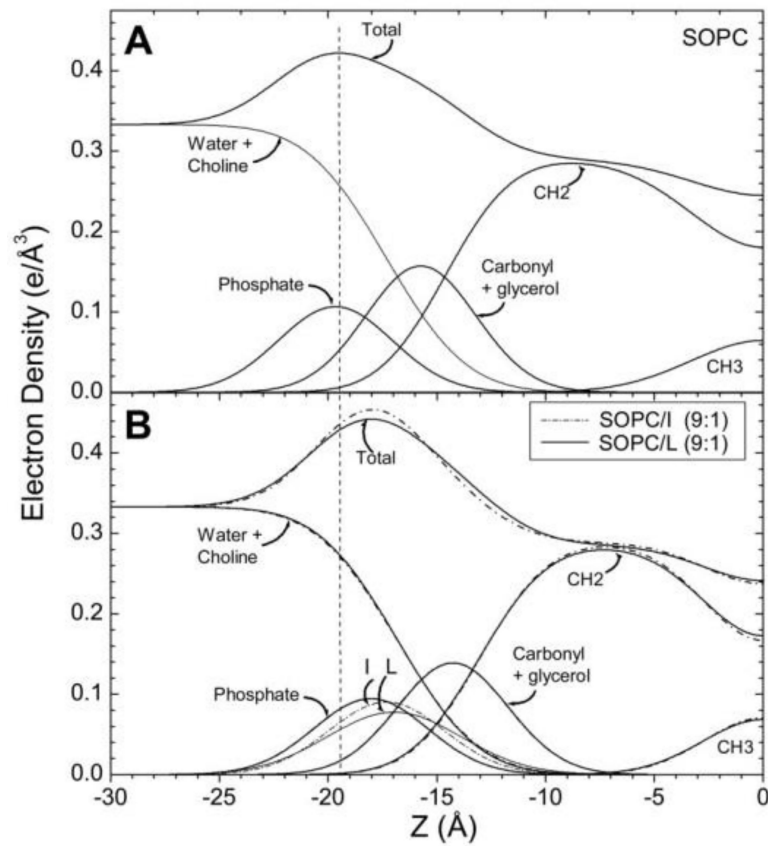


Figure 11. (right). Electron density profiles of SOPC (A) and SOPC/peptides (B) obtained using the H2 model with a Gaussian added for the peptides. Component groups are labeled and the thinning of the bilayer caused by either peptide is emphasized by the dotted vertical line. Figure from Ref. [5].

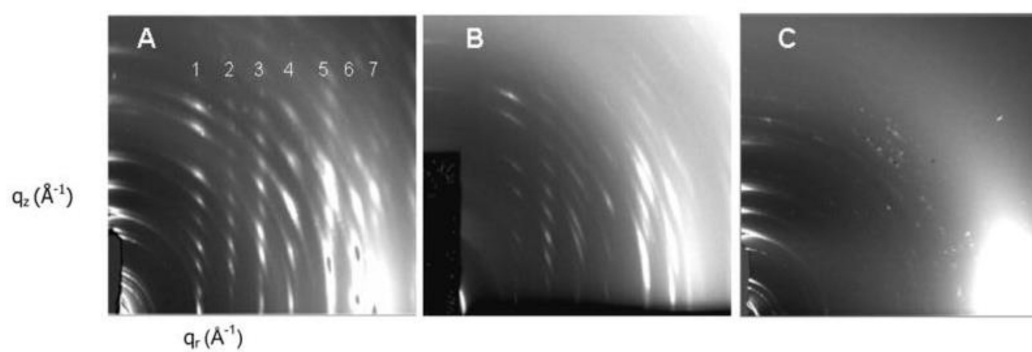


Figure 12. 2D CCD images of crystalline X-ray wide-angle diffraction patterns in the dried state of (A) SOPC/chol (5:5)/I (9:1), (B) SOPC/chol (5:5)/L (9:1), (C) SOPC/chol (5:5). Figure from Ref. 5.

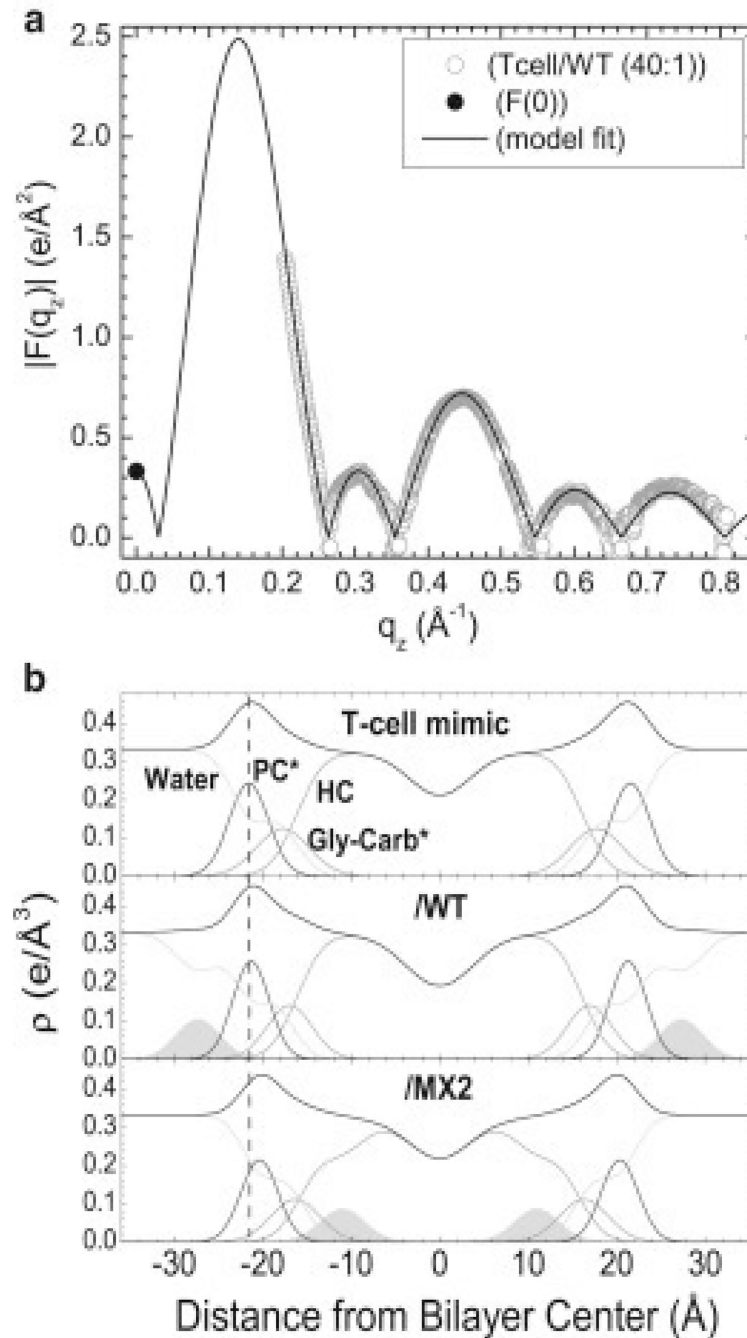


Figure 13.

(a) Model fit to $|F(q_z)|$ obtained from X-ray data and $F(0)$ obtained from volumetric data for T-cell/WT. (b) Electron density profiles for T-cell mimic control with WT and MX2 at 40:1 lipid:peptide molar ratio. The component groups are water, PC* (phosphocholine*), Gly-carb* (glycerol plus carbonyls*), HC (lipid hydrocarbon chains plus cholesterol), and LLP2 peptides (shaded). * indicates a weighted average group. Vertical dotted line facilitates comparison of membrane thickness. Figure from Ref. [15].

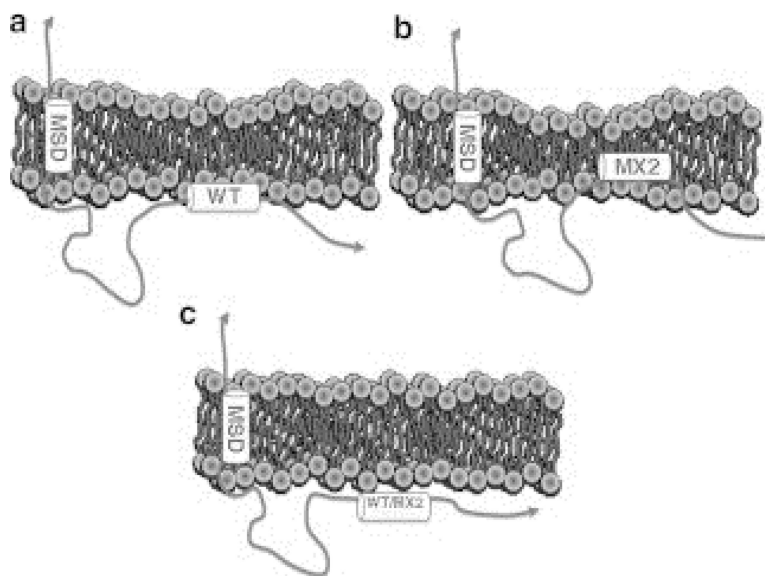


Figure 14. Cartoon showing differences in the interaction of LLP2 with T-cell and HIV membranes. a. WT LLP2 binds to the outer headgroup region and thins the T-cell membrane. b. MX2 binds more deeply into the hydrocarbon interior and thins the T-cell membrane to a greater extent. c. WT and MX2 LLP2 only weakly bind to the HIV virion membrane. MSD, membrane spanning domain. Figure from Ref. [15].

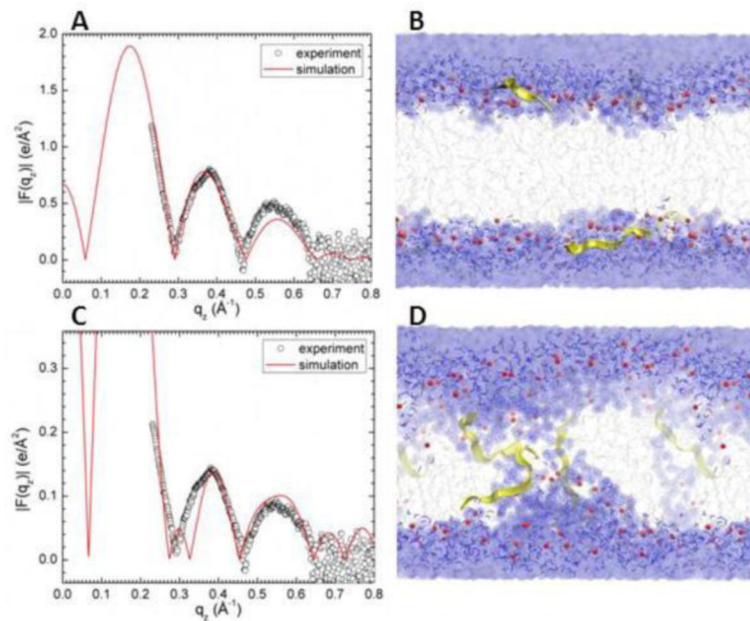


Figure 15. MD simulated form factors (red solid lines in A and C of Tat/DOPC+Tat, $x = 0.03$), with Tat fixed at $Z_{\text{Tat}} = 18 \text{ \AA}$ (A) and 5 \AA (C) from the bilayer center compared to experimental form factors (open circles). Corresponding snapshots are shown in B and D in which the lipid chains are represented as gray sticks on a white background, Tatts are yellow, phosphate groups are red and water is blue. Chi-square of the fit was 14.8 for A and 80.3 for C. Figure from Ref. [16].

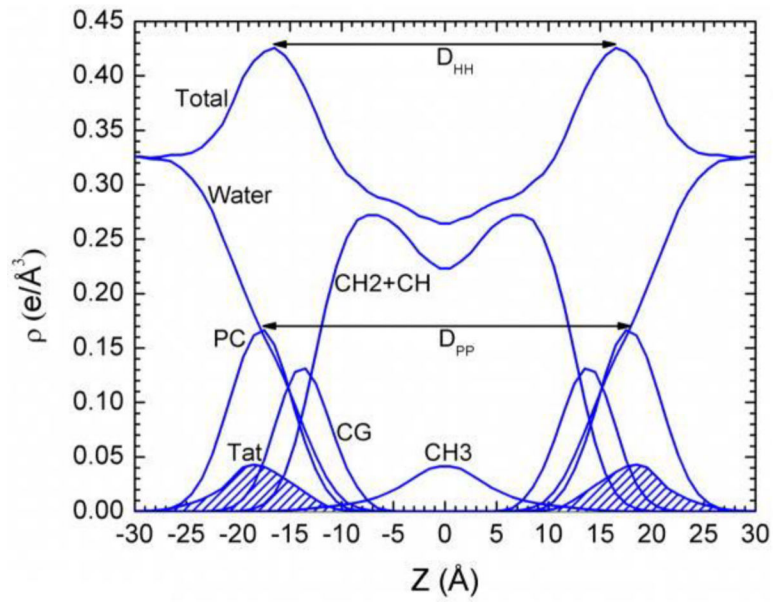


Figure 16.

Symmetrized total electron density profile (EDP) from the simulation with the form factor shown in Fig. 15A. Also shown are the component group contributions. Component groups are labeled: PC, phosphocholine; CG, carbonyl-glycerol; CH₂ + CH, methylene and methane hydrocarbon region; CH₃, terminal methyl; Tat peptide distribution is shaded. Figure from Ref. [16].

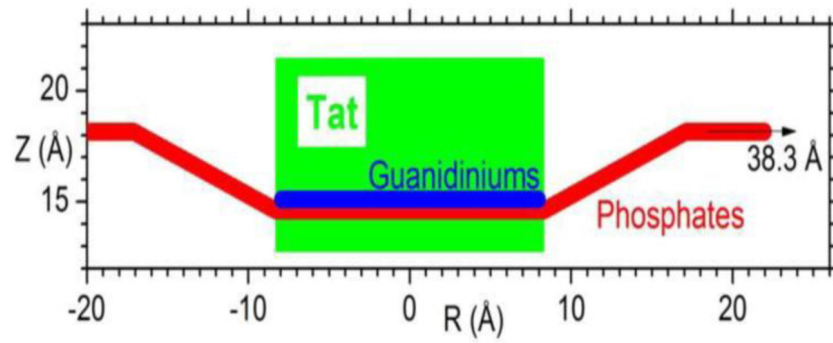


Figure 17.

Location of Tat in DOPC bilayer. Tat is represented as a cylinder, Z is the distance from the bilayer center, and R is the in-plane distance from the center of Tat. The average Z of the lipid phosphates as a function of R and the arginine guanidiniums are shown in red and blue respectively. Figure from Ref. [16].

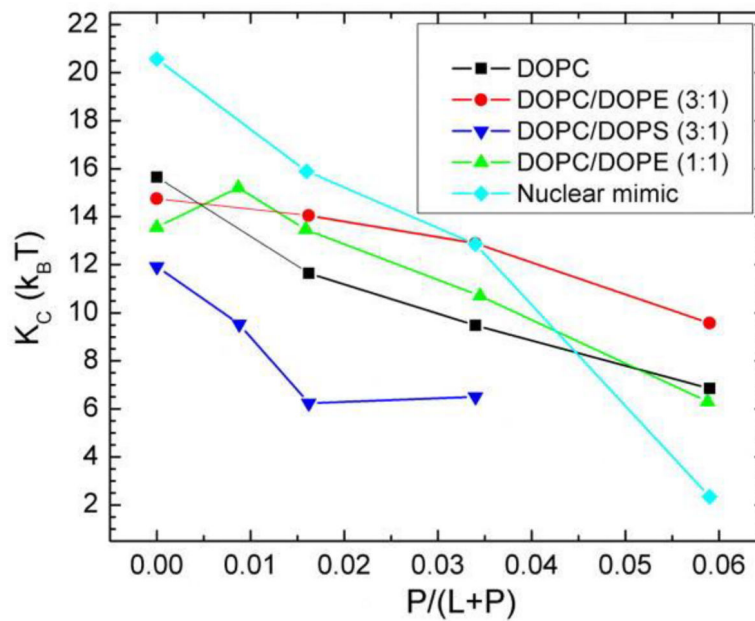


Figure 18. Bilayer bending modulus, K_C , vs. $P/(L+P)$ mole fraction. D-spacings for DOPC/Tat mixtures varied from 64 to 68 Å, for DOPC/DOPE/Tat mixtures from 64 to 69 Å, for DOPC/DOPS/Tat (3:1) mixtures from 57 Å to >100 Å (pure DOPS was unbound), and for nuclear mimic/Tat mixtures from unbound (control) to 64 Å. Estimated uncertainty in all values is ± 2 . Nuclear mimic is composed of POPC/POPE/POPS/SoyPI/Cholesterol 69:15:2:4:11 based on Ref. [90]. Figure from Ref. [16].

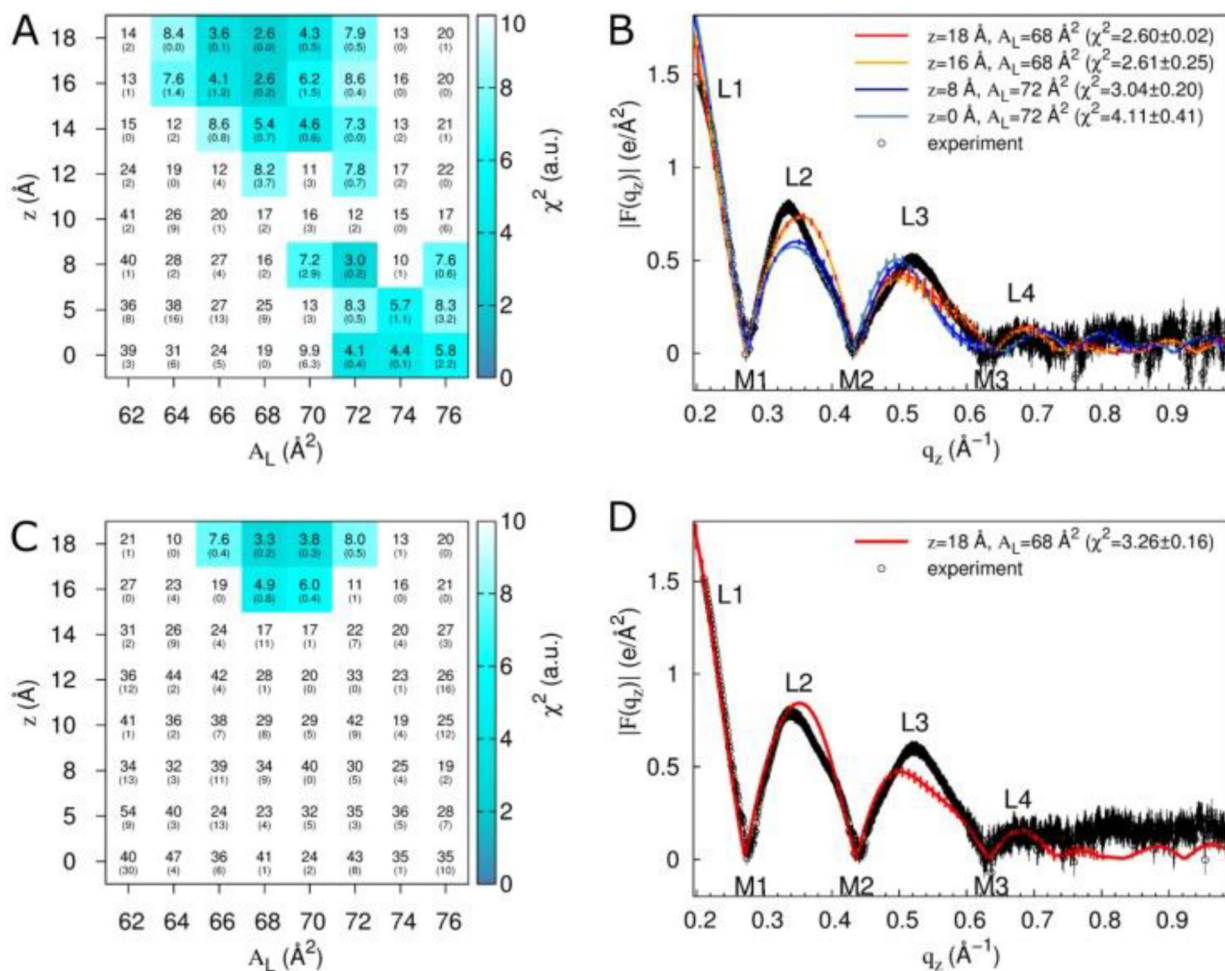


Figure 19.

Fits of simulation to experiment for a systematic evaluation of z_{Tat} and A_L in DOPC:DOPE (1:1). Data are from simulations with two (A,B) or four (C,D) molecules of Tat₄₇₋₅₇ (simulation mole fractions of 0.015 and 0.030, respectively; comparisons are to experimental data at mole fractions of 0.016 and 0.034, respectively). (A,C) Color and inset numbers represent χ^2 values (standard deviations of averages from repeat simulations in parentheses). (B,D) Selected form factors (χ^2 values in parentheses). Out-of-plane scattering lobes (L) and minima (M) are numbered based on experimental data, which are averaged over two (B) and nine (D) experiments. Black vertical lines depict experimental error estimates (see Methods). Vertical lines on the simulation data depict standard deviations between two starting configurations. Experimental data in (B) and (D) were scaled to the simulations at $z = 18 \text{ \AA}$, $A_L = 68 \text{ \AA}^2$. Figure from Ref. [17].

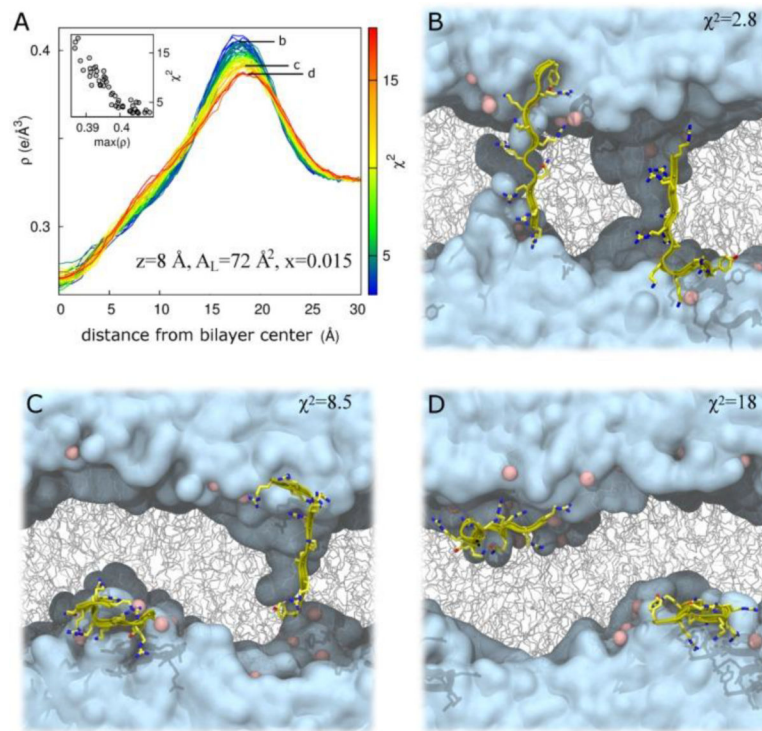


Figure 20.

Bilayer undulations increase χ^2 in DOPC:DOPE (1:1) at $z = 8 \text{ \AA}$, $A_L = 72 \text{ \AA}^2$ for a Tat₄₇₋₅₇ mole fraction of 0.015. (A) Total system electron density, ρ , as a function of absolute distance from the global bilayer center along its global normal for $N = 52$ simulations, colored by χ^2 value representing a range from best (blue) to worst (red) fit to experiment. Inset, χ^2 as a function of the maximum value of ρ . Labels b, c, and d in part A identify electron density curves for systems whose snapshots are shown in parts B, C, and D, respectively. (B–D) Snapshots after 100 ns of simulation in repeats with χ^2 values of 2.8 (B); 8.5 (C); and 18 (D). Water is cyan, lipids are gray, lipid headgroup phosphorus atoms are pink, and protein is yellow. Protein is rendered in front of all other molecules for clarity. Figure from Ref. [17].

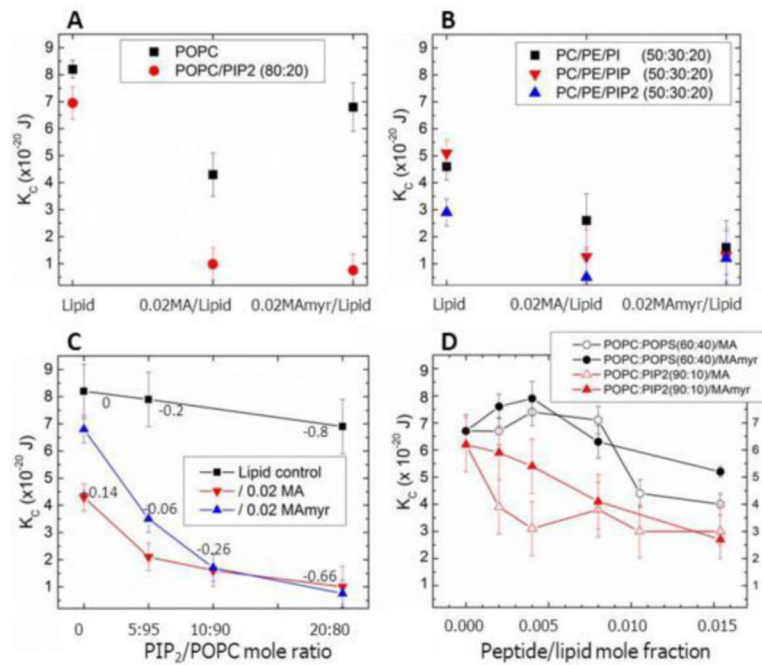


Figure 21.

Bending modulus, K_C , as a function of lipid and peptide concentration. (A) 0.02 MA₃₁ or MA₃₁myr in POPC, in POPC/PIP₂ (80:20), (B) 0.02 MA₃₁ and MA₃₁myr in POPC/POPE/PI (50:30:20), in POPC/POPE/PIP (50:30:20), in POPC/POPE/PIP₂ (50:30:20), (C) 0.02 MA₃₁ or MA₃₁myr in POPC with increasing PIP₂, (D) Increasing MA₃₁ or MA₃₁myr in POPC/POPS (60:40) or POPC/PIP₂ (90:10). Numbers in Fig. 21C indicate the net membrane charge/lipid plus protein. Lipid control indicates samples with no peptides. Error bars represent standard deviations of the average K_C values from 2 to 5 different samples. Figure from Ref. [18]. To compare K_C units in Joules to kT units, divide by 0.43×10^{-20} J.

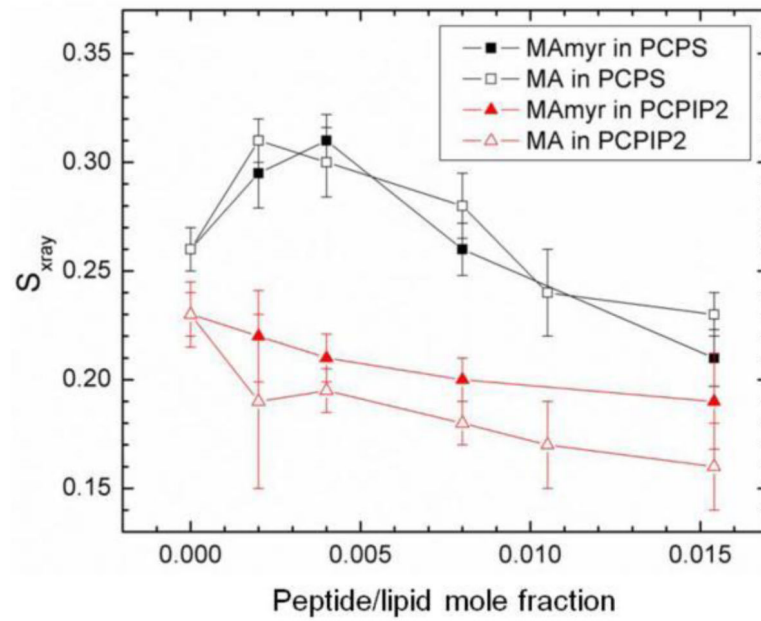


Figure 22.

S_{xray} order parameter for MA₃₁myr or MA in POPC/POPS (60:40), or POPC/PIP₂ (90:10). S_{xray} values were corrected for misalignment of layers. Error bars indicate standard deviations from the average S_{xray} from 2 or more images. Figure from Ref. [18].

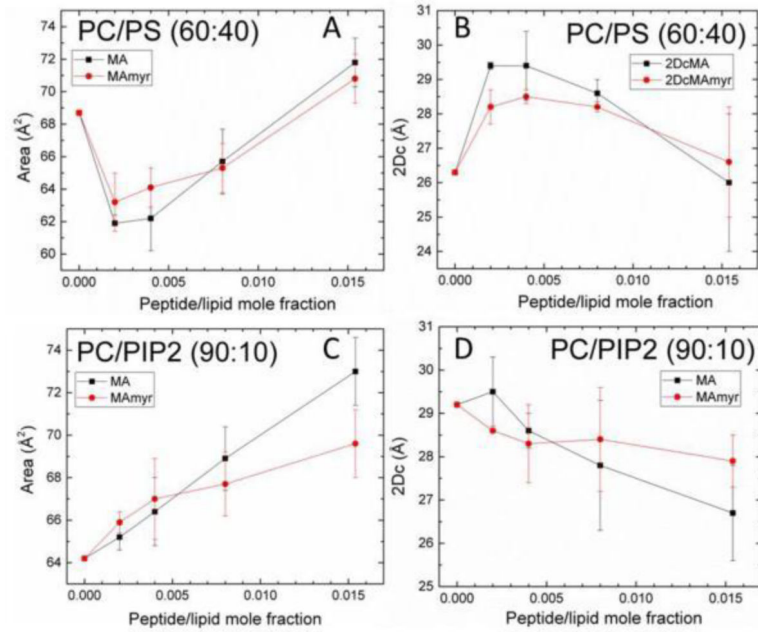


Figure 23.

Structural results vs. increasing peptide mole ratio: (A) area/lipid A_L in POPC/POPS (60:40), (B) hydrocarbon thickness $2D_C$ in POPC/POPS (60:40), (C) A_L in POPC/PIP₂ (90:10), (D) $2D_C$ in POPC/PIP₂ (90:10). Error bars indicate the standard deviations of the average of 2–5 fits. Figure from Ref. [18].

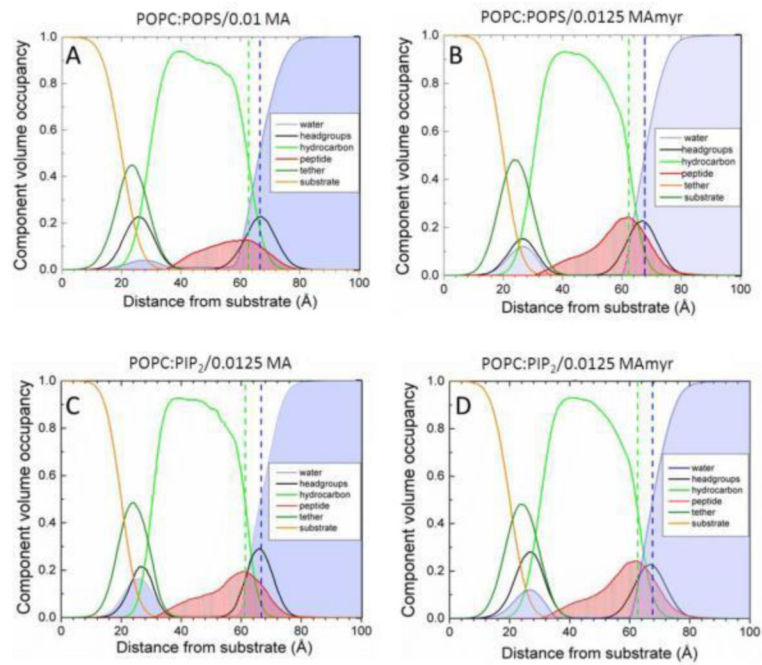


Figure 24.

Scattering density profiles obtained from neutron reflectivity. (A) POPC/POPS (60:40 mol ratio)/0.01 MA₃₁, (B) POPC/POPS (60:40)/0.0125 MA₃₁myr, (C) POPC/PIP₂ (90:10)/0.0125 MA₃₁, (D) POPC/PIP₂ (90:10)/0.0125 MA₃₁myr. Bilayer components are shown in the legends; Gibbs dividing surfaces HC (green dashed line) and water (blue dashed line) are shown. Figure from Ref. [18].

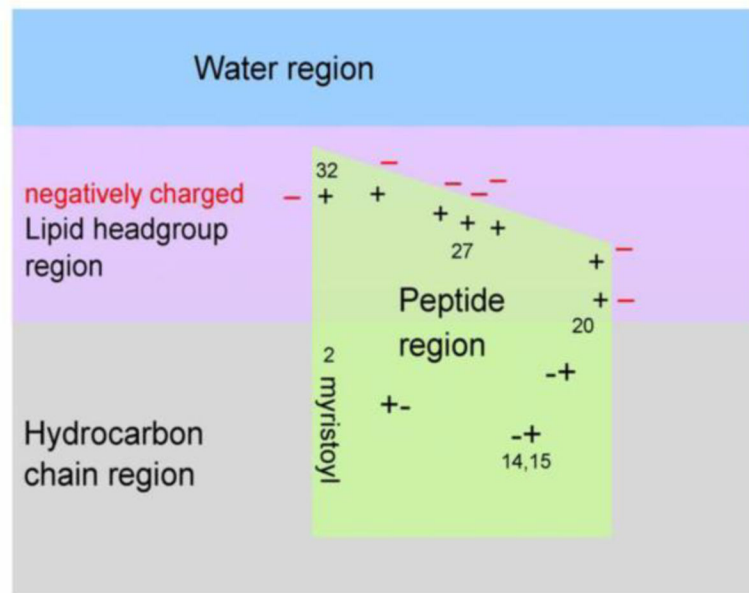


Figure 25.

Cartoon representation of MA₃₁myr insertion into a negatively charged membrane, based upon neutron reflectivity results. The location of the 7 positively charged amino acid residues in the HBR from AA20–32 are visualized by + signs with nearby negatively charged lipid headgroups (–signs). The charged amino acids in the AA2–19 stretch are visualized as +-pairs. The last AA32 is visualized near bulk water to allow the remainder of the MA₁₃₁protein to reside in water. Figure from Ref. [18].

Table1.Bending modulus K_C

Lipid	x_{Chol}	Peptide	x_{FP}	K_C/kT
LM3	0.33		0	32±2
LM3	0.33	FP23	0.048	5.3±0.3
LM3	0.33	FP23	0.091	4.4±0.3
LM3	0.33	FPtri	0.016	6.6±1.3
LM3	0.33	FPwsm	0.048	25±2
DOPC			0	19±0.9
DOPC		FP23	0.063	6.2±0.4
DOPC	0.3		0	18±0.4
DOPC	0.3	FP23	0.063	5.8±0.7
diC22:1PC			0	31±3
diC22:1PC		FP23	0.063	2.4±0.8
diC22:1PC	0.3		0	30±3
diC22:1PCl	0.3	FP23	0.063	2.0±0.8

Adapted from Ref. [15].

Table 2.

Radial and areal results for DOPE and 2 mol% FP23/DOPE

Sample	ϕ_w	D_{hex}	R_w	R_{0P}	R_s	R_c	R_1	A_w	A_p	A_1
DOPE Ref.[50]	0.30	63.8	21.1	28.5	—	—	—	49.6	64.2	—
DOPE	0.30	63.3	21.0	27.3	16.9	25.6	38.5	50.0	65.0	91.6
DOPE/FP23	0.28	62.3	20.0	25.6	15.8	24.5	37.8	50.1	64.2	94.5

Units are in the appropriate powers of Angstroms. Table from Ref. [6].

Table 3.

Structure and interaction results

Sample	K_C/kT	$2D_C$ (Å)	A^+ (Å ²)	Z_{pep}	S_{xray}
SOPC	21.5±2.8	29.2±0.4	67.0±0.9	---	0.33 ± 0.03
SOPC/I (9:1)	5.5±0.5	26.1	75.7	17.3	0.22 ± 0.02
SOPC/L (9:1)	10.0±1.0	26.2	75.8	17.0	0.24 ± 0.02
SOPC/Chol (7:3)	32.3±1.2 *	32.9 *	73.8±1.0 *	---	0.65 ± 0.06
SOPC/Chol (7:3)/ I (9:1)	5.7±3.1	29.1±1.5 **	109±5 **	10±0 **	0.39 ± 0.04
SOPC/Chol (7:3)/ L (9:1)	9.1±1.2	29.8±2.8 **	111±8 **	10.5±0.3 **	0.37 ± 0.04

Data from Ref. [5], unless otherwise noted.

* From Ref.[56]

** (previously unpublished)

+ area/unit cell (lipid+additive)

Table 4.

Lipid composition of membrane mimics

Lipid	Mole percentage		
	HIV	HIV-neg	T-cell
C 16 Ceramide	0.8	1.0	1.1
Soy Phosphoinositol (PI)	1.3	0	6.3
Brain PI(4)P	1.3	0	0.9
Brain PI(4,5)P2	1.3	0	1.1
12:0 diHydroSphingomyelin (diHSM)	1.7	1.8	2.7
PO-Phosphoethanolamine (POPE)	4.2	4.7	6.3
PO-Phosphoserine (POPS)	7.5	0	9.1
Plasmalogen PE	7.5	8.5	10.9
Egg Sphingomyelin (Egg SM)	14.2	16.0	16.3
PO-PhosphoCholine (POPC)	15.1	17.0	15.4
Cholesterol (Chol)	45.2	51.0	29.9

Table 5.

Bending and chain order results

Sample	T-cell mimic		HIV mimic		HIV-neg mimic		HIV extracted
	K_C	S_{xray}	K_C	S_{xray}	K_C	S_{xray}	K_C
Membrane	16.6	0.71	8.2	0.80	7.0	0.80	6.1
/WT(40:1)	12.6	0.72	8.2	0.79	7.0	0.78	6.8
/WT(20:1)	7.7	0.73	5.4	0.78	–	0.81	–
/MX2(40:1)	5.3	0.67	6.8	0.77	6.8	0.79	*
/MX2(20:1)	4.1	0.72	8.9	0.80	6.5	0.77	–

 K_C in kT units.

* This sample did not reach full hydration.

Table adapted from Ref. [15].

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