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Membrane Disruption Mechanism of a Prion Peptide (106-126) Investigated by Atomic Force Microscopy, Raman and Electron Paramagnetic Resonance Spectroscopy

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Abstract

A fragment of the human prion protein spanning residues 106-126 (PrP106-126) recapitulates many essential properties of the disease-causing protein such as amyloidogenicity and cytotoxicity. PrP106-126 has an amphipathic characteristic that resembles many antimicrobial peptides (AMPs). Therefore, the toxic effect of PrP106-126 could arise from a direct association of monomeric peptides with membrane matrix. Several experimental approaches are employed to scrutinize the impacts of monomeric PrP106-126 on model lipid membranes. Porous defects in planar bilayers are observed by using solution atomic force microscopy. Adding cholesterol does not impede defect formation. Force spectroscopy experiment shows that PrP106-126 reduces Young's modulus of planar lipid bilayers. We use Raman microspectroscopy to study the effect of PrP106-126 on lipid vibrational dynamics. For phosphatidylcholine lipids, PrP106-126 disorders the intra-chain conformation, while the inter-chain interaction is not altered; for phosphatidylethanolamine lipids, PrP106-126 increases the inter-chain interaction, while the intra-chain conformational order remains similar. We explain the observed differences by considering different modes of peptide insertion. Finally, electron paramagnetic resonance spectroscopy shows that PrP106-126 progressively decreases the orientational order of lipid acyl chains in magnetically aligned bicelles. Together, our experimental data support the proposition that monomeric PrP106-126 can disrupt lipid membranes by using similar mechanisms found in AMPs.

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Supporting Information

Figure S1, Raman spectra of deuterium water and PrP106-126; Figure S2, Time-dependent AFM images of a POPC+30mol% Chol bilayer exposed to 0.2 μ M PrP106-126; Figure S3, A typical force curve obtained from force spectroscopy experiment; Figure S4, AFM image of a POPC bilayer exposed to 0.5 μ M PrP106-126 at pH=4.6. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Aberrant conversion and accumulation of the prion protein (PrP) are responsible for the onset and progression of prion diseases, also known as transmissible spongiform encephalopathies (TSEs)¹. The pathogenic form of PrP is characterized by β -sheet enriched fibrillar structures that are partially resistant to enzyme degradation. The amyloidogenic propensity of PrP is recapitulated by a truncated form that spans residues 106-126 of human PrP (PrP106-126). The neurotoxic effect of PrP106-126 was first discovered two decades ago by chronically exposing cultured rat hippocampal neurons to peptide solutions containing PrP106-126². Later studies have corroborated the toxic effect of PrP106-126³⁻⁶, while others remain conservative⁷. Despite extensive studies, the molecular determinants of PrP106-126 (and the pathogenic form of PrP) that are responsible for nerve cell death are still largely unknown⁸⁻¹⁷. For example, what is the identity of the toxic species that forms along the aggregation pathway of PrP (and its fragments)? The ambiguity in toxic identity also renders difficulty in assessing molecular events that lead to nerve cell death. Based on the observation that the prion protein contains a glycosylphosphatidylinositol (GPI) anchor¹⁸, membrane interaction has been suggested as a key event in the pathology of prion diseases¹⁹⁻²³.

Primary sequence analysis shows that PrP106-126 exhibits an amphipathic characteristic that is commonly found in antimicrobial peptides (AMPs)²⁵⁻²⁶. In particular, the polar residues of PrP106-126 are segregated at the N-terminus, whereas the hydrophobic residues are localized at the C-terminus (Fig. 1). The amphipathic nature of PrP106-126 will likely promote peptide partition into membranous structures. Moreover, PrP106-126 has a polymorphic secondary structure that is dependent on environmental conditions²⁷⁻²⁸. The lack of a defined morphology may render the peptide more disruptive on membrane structural integrity²⁹. By incubating PrP106-126 at low pH, Walsh et al. prepared aged solutions containing monomers and aggregated species (i.e. oligomers and fibrils)³⁰. The addition of oligomer solutions inhibited bacterial proliferation, while fibrils exerted a negligible impact³⁰. Although it was not explicitly described in that paper, oligomer solutions used by the authors likely contained PrP106-126 monomers. Therefore, the observed antimicrobial activity could be attributed to oligomers and/or monomers. Walsh et al. also used atomic force microscopy (AFM) to observe transmembrane defects caused by oligomer solutions of PrP106-126³⁰. However, since no oligomers with a hydrodynamic radius of 30-40 nm (previously reported by the same group³¹) were visible in their AFM images, it is unclear whether the observed defects were caused by oligomers or other species such as monomers. Indeed, other groups have shown that porous defects do form when incubating monomeric forms of PrP106-126 with planar lipid bilayers³²⁻³⁵. Interestingly, another study showed that membrane interaction of PrP106-126 was weak under physiological conditions³⁶.

In this paper, we explore whether the monomeric form of PrP106-126 interacts with model lipid membranes at physiological pH. Moreover, if the interaction does occur, what are the consequences on physical properties of model lipid membranes? Our hypothesis is that the amphipathic nature of PrP106-126 bestows the peptide similar characteristics as AMPs against membranous structures. The impaired membrane integrity caused by monomeric

PrP106-126 may hamper cell viability, thus accounting for the reported toxicity toward bacteria and eukaryotic cells. We use planar lipid bilayers, vesicular dispersions, and magnetically aligned bicelles as model systems. To suppress the aggregation rate of PrP106-126, the C-terminal is amidated^{37–38}. The validity of C-terminal amidation is supported by the observation that the effect on primary neural cultures was not affected by the amidation³. Circular dichroism measurements also showed that PrP106-126 with free or blocked termini exhibited the same structure³⁹. Solution AFM is used to visualize the impact of PrP106-126 on lipid bilayer topographic structures. To obtain mechanical modulation on planar lipid bilayers, we perform AFM-based force spectroscopy measurements. Analysis of the obtained force spectroscopy data allows us to determine changes of the bilayer Young's modulus E as a function of the peptide concentration. To explore atomic-level interactions, we use Raman microspectroscopy. Modulations of lipid intra-chain conformation and inter-chain packing are determined by evaluating Raman spectral bands that are registered with various vibration modes. Finally, we use electron paramagnetic resonance (EPR) spectroscopy to study the effect of PrP106-126 on orientational order of lipid acyl chains in magnetically aligned bicelles. Our experimental results provide useful insights into the molecular mechanism used by monomeric PrP106-126 in impairing physical properties of lipid membranes.

Materials and Methods

Lipids including 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine (POPE), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), cholesterol (Chol), and cardiolipin 1',3'-bis[1,2-dioleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TOCL) are purchased from Avanti Polar Lipids (Alabaster, AL). 5-doxyl stearic acid (5-SASL), $\text{TmCl}_3 \cdot 6\text{H}_2\text{O}$, and VC (vitamin C) are purchased from Sigma-Aldrich (St. Louis, MO). C-terminal amidated peptide PrP106-126 ($\text{K}^{106}\text{TNMKHMGAAAAGAVVGGLG}^{126}\text{-NH}_2$) is synthesized by the solid synthesis method. For simplicity, we will refer to the peptide as PrP106-126 without explicitly noting the amidation. Peptide purity is 98% as indicated by analytical HPLC elution. Fresh solutions containing monomeric PrP106-126 are prepared by dissolving lyophilized peptide in 10 mM HEPES pH 7.4. Centrifugation is used to remove preexisting insoluble aggregates.

Solution AFM imaging

Lipid mixtures are prepared by mixing appropriate ratio of stock solutions in glass test tubes. Organic solvents are removed by a gentle stream of argon gas, followed by vacuum pumping for ~2 h. Lipid dry films are hydrated in 10 mM HEPES pH 7.4. Small unilamellar vesicles (SUVs) are produced by ultrasonication using a Sonic Dismembrator and a 3-mm microprobe. The obtained SUVs are centrifuged briefly before planar bilayer preparation. A Multimode 8 AFM (Bruker, Santa Barbara, CA) and a Nanoscope V controller are used for solution AFM imaging (room temperature). Experimental procedure has been reported elsewhere^{40–46}. Planar bilayers are formed by the vesicle rupture and fusion mechanism on a freshly cleaved mica film^{47–48}. Excess vesicles are flushed out after bilayer formation. The peak-force quantitative nanomechanics (QNM) mode is used for bilayer imaging. In addition

to bilayer topography, we also map peak-force error signal that is sensitive to topographic edges. Square images are acquired using a silicon nitride probe DNP-S10 with a scan rate of ~1 Hz. Height images are flattened by subtracting a second order polynomial background. Image analysis is performed using in-house Matlab scripts.

AFM-based force spectroscopy

Force spectroscopy measurements are performed at selected regions using the automated force-ramping function. A *z*-range of 100 nm and a tip speed of 200 nm/s are used for the measurements. For data analysis, only the force curve corresponding to the approaching direction is used. One parameter needed for force data analysis is the tip end radius. The nominal tip radius is 10 nm for the DNP-S10 probe. To obtain a more accurate estimation, we use a tip quantification function provided by the Nanoscope Analysis software.

Raman microspectroscopy

Lipid films (0.5 mg) of POPC or POPE are prepared from stock solutions. After vacuum drying, lipid films are hydrated by 100 μ L of deuterium water (99.8 atom % of D, Sigma-Aldrich, St. Louis, MO). A stock solution of monomeric PrP106-126 is prepared by using deuterium water as the solvent (0.4 mM). Lipid films are hydrated by mixing deuterium water and an aliquot of the peptide stock (total volume of 100 μ L), so the resulting peptide to lipid molar ratio is 1/20. Vesicular dispersions are homogenized by freeze/thaw cycles. For Raman measurements, lipid dispersions (30 μ L) are transferred to a sample chamber that is formed by placing a press-to-seal silicone sheet (0.25 mm thick, Grace Bio-Labs, Inc., Bend, OR) between two glass coverslips. Sample temperature (24°C for POPC and 30°C for POPE) is controlled by a Peltier module connected to a bipolar thermoelectric temperature controller (TC-720, TE Technology, Inc., Traverse City, MI).

Raman experiment is performed using a confocal microscope-based Raman spectrometer (LabRAM HR Evolution, Horiba Scientific). Lipid vesicles (diameter of ~10 μ m) immobilized on the proximal surface of the coverslip chamber (relative to the objective) are carefully adjusted to the confocal plane of a 532 nm laser beam (9 mW). Backscattered photons passing through a 50 $\times$ objective (NA 0.75 with a working distance of 0.38 mm) are filtered by a holographic notch filter before being detected by a Synapse back-illuminated deep depletion CCD (1024 $\times$ 256 pixels). To obtain various vibration modes, we measure wavenumbers ranging from 500 to 3300 cm^{-1} using 1800 gr/mm diffraction gratings. The resulting spectral resolution is 0.5 cm^{-1} . With an integration time of 20 s, the typical acquisition time for one spectrum is ~5 min. Due to variations such as the depth of the confocal plane within the lipid dispersion and the number of water molecules within the confocal scattering volume, the background arising from water scattering can vary between different measurements. This is illustrated by Raman spectra of the deuterium water at two depths (Fig. S1, Supporting Information). Based on this observation, the water background cannot be removed by simply subtracting a water spectrum. Instead, we use a multi-point baseline subtraction to remove water contribution⁴⁹.

Electron paramagnetic resonance (EPR) spectroscopy

EPR spectra are collected using a Bruker E680 spectrometer in X-band (9.5 GHz), equipped with a High Sensitivity cavity (ER 4119HS) and a variable temperature controller (ER 4111VT), all from Bruker BioSpin GmbH, Rheinstetten, Germany. The equipment is located at the National High Magnetic Field Laboratory (NHMFL). The spectra are acquired with 100 kHz modulation frequency, 3.2 mW incident microwave power (18 dB), 0.1 mT modulation amplitude, 20.48 ms time constant, 40.96 ms conversion time, and 15 mT scan width.

Lipids in chloroform are mixed and dried under a stream of nitrogen to obtain a thin film. The residual solvent is evaporated in a vacuum chamber overnight. The final lipid composition of each preparation is 5.6 μmol of DMPC, 1.55 μmol of DHPC, and 50 nmol of 5-SASL (0.7% of the total lipids). The Q ratio ($Q=[\text{DMPC}]/[\text{DHPC}]$) for these bicelles is 3.6. The PrP106-126 peptide is co-dried with lipids to obtain the desired peptide to lipid (P/L) ratios relative to the DMPC concentration. The dried film is rehydrated in 21.5 μL of 50 mM HEPES pH 7.0, and the suspension is repeatedly vortexed and sonicated in ice-cold water for ~ 15 min. The solution is then subjected to four freeze/thaw cycles. The resulting bicelle solution appears transparent and viscous.

We follow a reported procedure for aligning bicelles in the magnetic field⁵⁰. 5 μL of 288 mM $\text{TmCl}_3 \cdot 6\text{H}_2\text{O}$ is mixed with 20 μL of a bicelle/peptide solution for a final $\text{Tm}^{3+}/\text{lipids}$ molar ratio of 0.2. A glass capillary tube (0.9 mm I.D.) containing the mixed solution is placed in the EPR cavity at room temperature (295 K). Under a magnetic field of 1350 mT, the sample temperature is slowly raised to 318 K (about 15 min) and then slowly lowered to the final temperature of 308 K. This procedure ensures parallel alignment of the bicelle membrane normal (**n**) and the external magnetic field (**B**₀), so that lipid tails and the nitrogen p_z orbital of the nitroxide moiety are roughly parallel to **B**₀⁵¹.

Lipid orientational order perturbed by peptide binding is quantified by the orientational order parameter S_{20} ^{52–53}. To achieve this, we simulate and fit experimental EPR spectra using the MultiComponent program (<http://www.chemistry.ucla.edu/directory/hubbell-wayne-l>). The simulation strategy has been described elsewhere^{52, 54}. Briefly, the experimental spectra are simulated using magnetic and motional parameters maintained at constant levels for all P/L ratios, while the orientational order parameter S_{20} , calculated from the orienting potential coefficient c_{20} ⁵³, is varied. The starting principal values of the g tensor and the A tensor are taken from the literature^{55–56} and then fitted to obtain the final values of $g_{xx}=2.0090$, $g_{yy}=2.0060$, $g_{zz}=2.0025$, $A_{xx}=6.0$ G, $A_{yy}=6.0$ G, and $A_{zz}=33.0$ G. The principal values of the diffusion tensor are fitted to obtain values of $D_{xx}=D_{yy}=9.4 \cdot 10^{-6} \text{ s}^{-1}$ and $D_{zz}=470 \cdot 10^{-6} \text{ s}^{-1}$. Orientations of the principal axes of the diffusion tensor relative to the g tensor reference frame are $\alpha_D = \gamma_D = 0^\circ$ and $\beta_D = 33.5^\circ$. A non-zero value of the β_D angle has been determined previously^{52, 54}. It was attributed to the different chain lengths of DMPC (14 carbons long) and the spin label 5-SASL (18 carbons long). The orientation between the diffusion frame and the director, here identified as parallel to the membrane normal, is set to $\psi=0^\circ$. Once all the parameters have been optimized, the orienting potential

coefficients are fitted (only c_{20} is employed for uniaxial symmetry) and the orientational order parameter S_{20} is subsequently calculated in MultiComponent.

Results

Solution AFM imaging

We perform solution AFM measurements to visualize PrP106-126-induced defects in mica-supported planar bilayers composed of POPC with 0, 30, and 50 mol% Chol. After removing excess SUVs, the height image of an intact POPC bilayer is shown in Fig. 2A. The control bilayer exhibits a smooth topographic structure with a height variation of ~ 0.1 nm. After image acquisition of the control bilayer at several locations, the AFM tip is retracted by ~ 22 μm , and a solution containing 0.2 μM PrP106-126 is injected into the AFM fluid cell. The POPC bilayer is allowed to react with the peptide solution for ~ 10 min, after which the AFM tip approaches the bilayer surface to acquire the modified bilayer topography. After tip engagement, sporadic defects are immediately observed (Fig. 2A). The corresponding height probability distribution shows that the defect depth is 3.4 nm (Fig. 2D). Image analysis is used to determine defect properties. Specifically, pixels with intensities smaller than the bilayer are assigned as defect pixels. Surface area fraction of the defects are obtained by calculating the total number of defect pixels. We find that the surface area fraction of defects induced by 0.2 μM PrP106-126 is 3%. To estimate defect size, we calculate the area occupied by each defect. (Defect areas are determined by using Matlab function `regionprops`.) By assuming that defects are circularly shaped, we obtain an average defect radius of 300 nm, which is much larger than that of canonical transmembrane pores. The total reaction time for the POPC bilayer with 0.2 μM PrP106-126 is ~ 1.5 h, during which the size and area fraction of the defects do not evolve drastically. To explore the effect of PrP106-126 at higher concentrations, we retract the AFM tip and inject a solution containing 0.5 μM PrP106-126 to replace the 0.2 μM solution. After incubation for 30 min, the peptide-induced defects become larger. Specifically, the surface area fraction and the average radius of the modified defects become 16% and 580 nm, respectively. Despite the marked change of the defect morphology, the defect depth remains at 3.4 nm as indicated by the height probability distribution (Fig. 2D).

Chol is known to modulate structural and mechanical properties of lipid membranes. Treatment with Chol has been shown to reduce the toxicity of PrP106-126 toward neuronal cell cultures⁵⁷. To study the effect of Chol on membrane defects induced by PrP106-126, we prepare POPC planar bilayers containing 30 mol% Chol (Fig. 2B). The addition of 0.2 μM PrP106-126 induces defects that are similar in size (average radius of 270 nm) and total area fraction (4%) compared to those of the POPC bilayer. One noticeable difference is that the defect depth increases to 4.2 nm in the presence of the 30 mol% Chol (Fig. 2E). Over the course of the ~ 1.5 -h incubation period, the peptide-induced defects do not evolve significantly. This is illustrated by a series of four images acquired at different time points over the incubation period (Fig. S2, Supporting Information). Increasing the peptide concentration to 0.5 μM results in a larger defect radius (480 nm) and a larger surface area fraction (18%), while the defect depth remains the same (4.2 nm). Interestingly, a few high-

rise regions are observed near defect edges. Height probability distribution shows that the high-rise regions protrude by 3.4 nm above the bilayer surface.

We also study the effect of 50 mol% Chol on membrane defects induced by PrP106-126 (Fig. 2C). The addition of 0.2 μM PrP106-126 elicits defects that have an average radius of 590 nm and an area fraction of 6%. A few high-rise regions are also observed at defect edges. Height probability distribution shows that for the POPC+50 mol% Chol bilayer, the average defect depth is 4.3 nm and the average height of the high-rise regions is 3.1 nm (Fig. 2F). Increasing peptide concentration to 0.5 μM causes larger defects with an average radius of 820 nm and an area fraction of 23%, while the defect depth remains at 4.3 nm. A few high-rise regions are also observed with an average height of 3.1 nm.

AFM-based force spectroscopy

We use AFM-based force spectroscopy to characterize the effect of monomeric PrP106-126 on mechanical properties of POPC/Chol bilayers. The experimental procedure for force spectroscopy measurements is similar to AFM imaging. We first prepare a control bilayer. After performing force spectroscopy measurements, we inject a peptide solution with incremental concentrations (i.e. 0.2 and 0.5 μM) into the AFM fluid cell. At each peptide concentration, force spectroscopy data are obtained at bilayer regions that do not contain visible defects.

It has been shown that the AFM force F can be described by a modified Hertz model^{58–59}:

$$F = \frac{16}{9} E R^{1/2} \delta^{3/2} \left(1 + 0.884\rho + 0.781\rho^2 + 0.386\rho^3 + 0.0048\rho^4 \right), \quad (1)$$

where R is the tip end radius, δ is the indentation depth of the AFM tip into a supported bilayer, and E is Young's modulus. δ can be calculated by the difference between the bilayer surface position s_0 and the tip position s : $\delta = s_0 - s$. (Positions s_0 and s are relative to the mica surface.) The dimensionless parameter ρ is defined by $\rho = \sqrt{R\delta}/h$, where h is the bilayer thickness. To evaluate h , we assume that the thin water layer residing between the mica surface and the planar bilayer has a thickness d_{WL} of 2 nm. The bilayer thickness h is then related to the bilayer surface position s_0 by $s_0 = h + 2$ nm. For each force curve, we determine the best set of E and s_0 by minimizing the difference between the experimental data and the theoretical prediction based on Eq. (1). An example of data fitting using the modified Hertz model is shown in Fig. S3 (Supporting Information). By analyzing all the force curves shown in Fig. 3, we obtain an average Young's modulus E of 59 MPa and an average bilayer surface position s_0 of 6.5 nm for the POPC bilayer. Our determined E value is consistent with reported values derived from studies using supported bilayers^{59–62}. It is noteworthy that model analysis using Eq. (1) implicitly assumes that the water layer underneath the planar bilayer is rigid⁶². This assumption is valid since the bulk modulus of water is ~ 2 GPa⁶³, much larger compared to those of lipid bilayers.

Two criteria are applied to choose the region for our force data analysis. The smallest force F_{min} is set to 0.3 nN to eliminate contributions from non-steric interactions (e.g.

electrostatic, van der Waals, and hydration forces) between the bilayer and the tip. Decreasing $F_{\min}$ to 0.1 nN reduces E by ~ 1 MPa. As the force F increases, the thin bilayer is elastically deformed by steric interaction with the tip. Our experimental data show that the force can deviate from elastic behavior before reaching the bilayer puncture force F_p – the maximum force that a bilayer can withstand before being punctured by the tip. This is especially true for bilayers exposed to PrP106-126. To eliminate the influence of the non-elastic behavior near bilayer puncture, we limit the largest force $F_{\max}$ to 90% of F_p . We also performed data analysis by setting $F_{\max}$ to 100% of F_p . The largest decrease of E (~ 3 MPa) is observed for bilayers treated with 0.5 μM peptide.

The absolute value of E derived from Eq. (1) is affected by the value of the tip end radius R^{62} . This partially explains the variation of E reported by different studies using AFM measurements^{59–62}. For the studies presented in this paper, the same probe is used to perform all the force spectroscopy measurements. This approach enables us to unambiguously determine the relative changes of bilayer nanomechanics induced by Chol and PrP106-126.

Incrementally adding 0.2 and 0.5 μM PrP106-126 to the POPC bilayer reduces the bilayer puncture force F_p by 0.3 nN. In parallel, the bilayer Young's modulus E decreases to 55 and 53 MPa, respectively. Although the changes of the mean values of E are comparable to experimental uncertainties (Table 1), two-sample t-test indicates that the p -values are $< 10^{-5}$, suggesting that the observed decreases of E at elevated peptide concentrations are significant. The bilayer surface position s_0 increases mildly by 0.1 nm (Fig. 4 and Table 1). Bilayer thickness h is defined by the difference between s_0 and the water layer thickness d_{WL} . The insensitivity of s_0 highlights that PrP106-126 does not markedly alter the thickness of the POPC bilayer. Using force spectroscopy measurements, we have observed a similar resilience of the bilayer thickness to the treatment of an antimicrobial peptidomimetic⁴⁶. Interestingly, introducing ethanol to mica-supported planar bilayers caused a stark decrease of the bilayer thickness as indicated by the shifting of force curves toward smaller s^{59} .

The effect of Chol on physical properties of the POPC bilayer is illustrated by the force spectroscopy data (Fig. 3B,C). Compared to the POPC bilayer, the bilayer puncture force F_p increases to 3.2 and 3.4 nN at 30 and 50 mol% Chol, respectively. Since F_p reflects the mechanical stability that a bilayer can withstand, the larger F_p highlights that Chol enhances bilayer mechanical integrity. Data fitting using the modified Hertz model shows that the Young's modulus E increases by 3 and 26 MPa at 30 and 50 mol% Chol, respectively. The observed increases of E are consistent with the role of Chol in enhancing bilayer rigidity^{64–65}. In addition to E , our data analysis shows that the bilayer surface position s_0 increases by 0.5 and 1.0 nm at 30 and 50 mol% Chol, respectively. The obtained increases of s_0 are in line with the bilayer thickening effect of Chol⁶⁶. Together, our force spectroscopy data recapitulates several known impacts of Chol on bilayer physical properties.

Force spectroscopy data of POPC+30 and 50 mol% Chol bilayers exposed to incremental concentrations of PrP106-126 are shown in Fig. 3B,C. Using model analysis based on Eq. (1), we determine the impacts of PrP106-126 on POPC/Chol bilayers. The resulting parameters are listed in Table 1. Several conclusions can be drawn based on the determined

parameters. Firstly, the largest changes in bilayer properties are obtained at 0.2 μM PrP106-126; increasing the peptide concentration to 0.5 μM does not cause drastic changes. Secondly, PrP106-126 decreases the bilayer Young's modulus E and the bilayer puncture force F_p for each bilayer. Thirdly, PrP106-126 slightly increases the surface position s_0 for each bilayer. Fourthly, the presence of Chol does not negate the reduction of bilayer mechanical properties caused by PrP106-126.

Raman microspectroscopy (POPC)

We use a Raman spectrometer coupled with a confocal microscope to probe the effect of monomeric PrP106-126 on lipid intra-chain conformation and lateral inter-chain interactions. Figure 5 shows the vibrational Raman spectrum of POPC dispersions at 24°C. The 1800-2760 cm^{-1} region is not displayed due to the lack of absorption bands. Each spectral interval is normalized by its maximum intensity. In the low-wavenumber region, the two bands at 715 and 768 cm^{-1} are caused by stretching vibrations of the choline and O-P-O moiety, respectively. The stretching vibration of skeletal C-C bonds of lipid acyl chains is reflected by the complex bands located at 1040-1140 cm^{-1} . Specifically, the two bands centered near 1063 and 1122 cm^{-1} correspond to the C-C *trans* configuration and the band centered near 1085 cm^{-1} is assigned to the C-C *gauche* rotation⁶⁷. Since the three bands are particularly sensitive to the C-C conformation of lipid acyl chains, the corresponding vibrational motions are referred to as skeletal optical modes (SOM). Studies of DPPC dispersions at different temperatures have shown that the gel-fluid phase transition of DPPC acyl chains is accompanied by an increase in the intensity ratio of the *gauche* to *trans* band⁶⁷⁻⁶⁸. The two bands centered near 1300 and 1438 cm^{-1} are assigned to the out-of-plane twisting and in-plane scissoring vibration of C-H bonds in CH_2 , respectively. The band centered near 1654 cm^{-1} is caused by stretching vibration of the C=C bond in the oleoyl chain of POPC. The C=O bond of POPC located at the glycerol-carbonyl backbone causes a broad band centered near 1730 cm^{-1} .

The congested region of 2800-3000 cm^{-1} is dominated by stretching vibrations of C-H bonds in methylene and methyl groups of lipid acyl chains. The intense bands centered near 2850 and 2888 cm^{-1} are assigned to symmetric and asymmetric C-H stretching vibration in methylene groups, whereas the weak bands centered near 2929 and 2959 cm^{-1} are assigned to symmetric and asymmetric C-H stretching vibration in methyl groups⁶⁹. Importantly, the intensity ratio of I_{2889}/I_{2850} is correlated with the inter-chain lateral packing/interaction. For example, the gel-fluid phase transition (or disruption of lateral chain packing) of phospholipid bilayers is associated with a decrease of the intensity ratio of I_{2889}/I_{2850} ⁶⁸.

The addition of PrP106-126 (P/L=1/20) causes marked changes of the SOM bands of POPC dispersions. The *trans* bands centered near 1063 and 1122 cm^{-1} become two shoulders that are superimposed on the *gauche* band centered near 1087 cm^{-1} . Using the *trans* band near 1063 cm^{-1} as a reference, the intensity ratio of I_{1085}/I_{1063} increases from 1.07 to 1.34. The observed increase indicates that PrP106-126 enhances the number of *gauche* rotations and decreases the probability of *trans* configuration of POPC acyl chains. Note that we cannot use the *trans* band near 1122 cm^{-1} as the reference since the band is not well resolved in the presence of the peptide. A similar increase in the intensity ratio of the *gauche* and *trans* band

was observed at elevated temperatures for DPPC dispersions; the increase became more pronounced when DPPC undergoes a gel-to-fluid phase transition⁶⁷. Gaber and Peticolas used the intensity ratio of the *trans* and *gauche* bands to describe the probability of the *trans* conformation of skeletal C-C bonds⁶⁸. Based on their notion, our observed changes of the SOM bands suggest that PrP106-126 induces conformational disorder of the skeletal C-C bonds of POPC acyl chains.

In contrast to the marked changes of the SOM bands, the sharp bands corresponding to the bending motions of CH₂ (i.e. 1300 and 1438 cm⁻¹) and the stretching motion of C=C (i.e. 1654 cm⁻¹) remain virtually the same after adding PrP106-126. Interestingly, the peptide augments the C=O band intensity at 1730 cm⁻¹. The stretching vibration of C=O at the interface of lipid headgroup and acyl chains is dependent on the environment near the backbone⁷⁰⁻⁷². The increase of the C=O band intensity highlights that PrP106-126 interacts with the glycerol-carbonyl backbone and modulates the stretching vibration of the C=O bond.

The lateral packing order of lipid acyl chains can be assessed by the principal bands of C-H stretching in the high-wavenumber region. Gaber and Peticolas used the intensity ratio of the asymmetric and symmetric stretching bands of C-H in methylene (i.e. I₂₈₈₈/I₂₈₅₀) to calculate a lateral order parameter: $S_{\text{lateral}} = (I_{2888}/I_{2850} - 0.7)/1.5$, which can qualitatively account for inter-chain vibrational coupling/interaction⁶⁸. (The two constants, 0.7 and 1.5, in the expression of S_{lateral} were used to eliminate contributions other than inter-chain lateral interaction.) Our calculated S_{lateral} is 0.32 for POPC dispersions. Adding PrP106-126 to POPC dispersions causes no discernable changes of the C-H stretching bands; therefore, our Raman data suggest that the lateral interaction of POPC acyl chains is not markedly perturbed by PrP106-126.

Raman microspectroscopy (POPE)

We also use Raman microspectroscopy to study interactions between PrP106-126 and POPE dispersions. The gel-to-fluid phase transition temperature of POPE is ~25°C. Since we are interested in POPE bilayers in the fluid phase, Raman measurements of POPE are performed at 30°C. The resulting POPE spectrum is shown in Fig. 6. Compared to POPC, POPE does not contain the sharp band at 715 cm⁻¹ that corresponds to stretching vibration of the choline group. Other differences also exist for weak bands located below 1000 cm⁻¹. For the three SOM bands, the intensity ratio of the *gauche* and *trans* bands is slightly smaller for POPE (I₁₀₈₀/I₁₀₆₄=0.96) than for POPC (I₁₀₈₅/I₁₀₆₃=1.07). Sharp bands for POPE that correspond well to those for POPC are observed at 1299, 1438, and 1654 cm⁻¹. In addition, two shoulders emerge near 1267 and 1456 cm⁻¹ in the POPE spectrum. Compared to POPC, POPE exhibits a larger intensity ratio of the C=C stretching band (1654 cm⁻¹) to the CH₂ twisting band (1438 cm⁻¹), i.e., 0.43 versus 0.30. The difference may signify a different conformational state of the C=C bond in POPE and POPC bilayers. POPE and POPC dispersions also exhibit a large difference in the C-H stretching region. The lateral order parameter S_{lateral} is 0.14 for POPE and 0.32 for POPC. The difference is evident by comparing band intensities near 2886/2888 and 2850 cm⁻¹. The smaller lateral order

parameter for POPE suggests that the inter-chain interaction is weaker in POPE than in POPC bilayers.

Adding PrP106-126 to POPE dispersions (P/L=1/20) does not noticeably modify the relative intensities of the SOM bands of POPE (Fig. 6). This result is in large contrast to POPC, whose *gauche* band is significantly enhanced compared to the *trans* bands. The insensitivity of the SOM bands indicates that PrP106-126 does not alter the skeletal C-C conformation of POPE acyl chains. Different effects of PrP106-126 on POPE and POPC lateral packing are observed in the C-H stretching region. Contrary to a decrease of the lateral order parameter S_{lateral} of POPC, PrP106-126 increases S_{lateral} of POPE from 0.14 to 0.24. The larger S_{lateral} induced by PrP106-126 indicates that the lateral interaction between POPE acyl chains is enhanced in the presence of the peptide.

Electron Paramagnetic Resonance (EPR) spectroscopy

EPR measurements are performed to determine how PrP106-126 perturbs lipid chain order in membrane-mimetic DMPC/DHPC bicelles. A trace amount of the spin label 5-SASL is present in bicelles. Interactions of the nitroxide moiety of 5-SASL with bicellar lipids modulate the EPR signal. EPR spectrum of the aligned bicelles without peptide shows three broad peaks that are typical for a nitroxide partially oriented in the magnetic field (Fig. 7A). Using simulation analysis, we find an orientational order parameter S_{20} of 0.439. Similar values were reported by other groups using EPR spectroscopy^{52, 54}. The addition of PrP106-126 at low P/L ratios results in noticeable changes of the spectral line shapes (i.e. 1/300 and 1/200). Specifically, a kink emerges near 332 mT and the peak near 331 mT becomes weaker. The peak near 331 mT is further suppressed at higher P/L ratios (i.e. 1/75 and 1/50). Similar suppression is observed for the broad peak near 336 mT as a function P/L. Numerical values of the orientational order parameter S_{20} for PrP106-126 and two reported peptides, alamethicin and magainin 2^{52, 54}, are displayed in Fig. 7B. Alamethicin is a representative of the peptaibol family and it is known to form voltage-dependent barrel-stave pores in lipid membranes⁷³. The S_{20} value for alamethicin decreases slowly, suggesting the existence of a high percentage of the chain order even at P/L=1/20 (S_{20} 0.25). In contrast, magainin 2 is well-known to cause extensive membrane disruption by forming toroidal pores and, at higher concentrations, acting as a detergent^{74–76}. The addition of magainin 2 to bicelles causes a rapid loss of the chain order even at low P/L ratios, with the orientational order parameter S_{20} quickly dropping to a value of 0.1 and below.

Compared to alamethicin and magainin 2, PrP106-126 causes an intermediate change of S_{20} . At low P/L ratios (i.e. 1/300 and 1/200), the S_{20} values for PrP106-126 are comparable to those for alamethicin. This suggests that at low P/L ratios, PrP106-126 does not induce severe disordering of lipid chains. At elevated P/L ratios (i.e. 1/75), however, there is a quick drop in S_{20} , which rapidly decreases to below 0.1. The isotropic limit of $S_{20}=0$ is almost achieved at P/L=1/50. The progressive decrease of the orientational order parameter S_{20} as a function P/L highlights that PrP106-126 disorders lipid chain conformation in membrane-mimetic bicellar structures.

Discussion

Solution AFM imaging

We use solution AFM to image membrane topography modulated by the amphipathic peptide PrP106-126. The addition of the monomeric peptide induces defects in POPC/Chol bilayers. Similar defects caused by PrP106-126 have been reported^{32–35}. By using an aged peptide solution incubated at low pH, Walsh et al. observed similar defects in planar bilayers³⁰. The authors attributed the cause to soluble oligomers formed by PrP106-126. However, since no 30–40 nm-sized oligomers³¹ were present in their AFM images, the causative entity for defect formation could be monomeric peptides³⁰. The depth of our obtained defects is 3.4 nm in POPC bilayers. This value is smaller than the total thickness of the planar bilayer and the water layer underneath the bilayer. One possibility is that the defects are only formed at the distal leaflet (relative to the mica surface) of the mica-supported bilayer. However, the monolayer thickness of a POPC bilayer is 2.1 nm⁷⁷, which is smaller than the defect depth; therefore, it is unlikely that the observed defects only transverse one leaflet of the planar bilayer. A plausible scenario is that the defects span the entire bilayer; however, the tip does not reach the mica surface during AFM imaging. This explanation is highly probable considering the repulsive interaction between the mica film and the AFM tip when they are close by^{35, 78}. Overall, our observation of transmembrane defects is consistent with earlier electrophysiology studies showing that PrP106-126 forms ion-permeable channels in model lipid bilayers^{79–80}.

The porous defects induced by PrP106-126 have an average radius larger than 200 nm. Using solution AFM imaging, we have obtained defects of ~5-nm-radius caused by the antimicrobial peptide melittin⁴⁵. Since transmembrane pores are typically smaller than 10 nm, the large defect sizes caused by PrP106-126 imply that the peptide does not act by forming canonical peptide-lined pores⁸¹. Instead, we speculate that PrP106-126 disrupts lipid bilayers by extracting lipid molecules from the bilayer environment. This proposition is supported by the observation of similar defects when planar bilayers are exposed to the nonionic detergent Triton X-100⁴⁵. Micron-sized defects have also been observed when mica-supported bilayers are exposed to antimicrobial peptides⁸². Based on the observation that defects can contiguously grow, the authors concluded that large-sized defects are caused by lipid removal from the mica surface⁸². Interestingly, the reported defects have a similar depth (~3 nm) as ours⁸².

In addition to porous defects, we observe a few high-rise regions that have a height of 3.1–3.4 nm in POPC/Chol bilayers. High-rise regions induced by PrP106-126 have also been observed by other groups³³. However, the reported high-rise regions have a much larger area coverage than ours^{32–33}. The difference is likely caused by different buffer conditions. For example, substantial area coverage (~26%) of the high-rise regions is observed when we expose a POPC bilayer to 0.5 μ M PrP106-126 at pH 4.6 (Fig. S4, Supporting Information). Using fusogenic calcium ions, we have shown that lipid molecules extracted from planar bilayers can form a second layer on top of the original bilayer⁴⁶. The process of forming a second layer is reminiscent of the formation of a mica-supported bilayer. In the later case, small vesicles are ruptured on a mica surface; ruptured patches fuse together to form a

supported bilayer^{47–48}. Based on the large sizes of defects, we have concluded that PrP106-126 induces porous defects by lipid extraction/dissolution. In the absence of fusion promoters, the extracted lipids will not spontaneously fuse onto existing surfaces. However, the fusion propensity of lipid molecules is enhanced at lower pH^{83–86}. This explains the formation of extensive high-rise regions in POPC bilayers at pH 4.6 (Fig. S4), but not at pH 7.4 (Fig. 2). Interestingly, PrP106-126 was found to exhibit a fusogenic property by promoting lipid mixing¹⁵. Since we do not observe many high-rise regions at pH 7.4, the fusogenic property of PrP106-126 is likely weak. Compared to the POPC bilayer, bilayers containing Chol contain more high-rise regions (Fig. 2). This observation conforms to various biophysical data showing that Chol plays a major role in membrane fusion⁸⁷.

To further support our conjecture that the high-rise regions are caused by the fusion of extracted lipids, we add 0.5 μ M PrP106-126 to a planar bilayer formed by POPC+10 mol% TOCL. It has been shown that membrane fusion can be promoted by anionic lipids⁸⁸. Indeed, we find that the presence of 10 mol% TOCL causes a large area coverage (~21%) of the high-rise regions near defect edges (Fig. 8A,B). Moreover, we observe many particle-like features deposited on the bilayer surface. To obtain a detailed view of the globular particles, we use AFM-based peak-force error mapping, which is a sensitive technique for edge detection (Fig. 8C,D). By correlating the height and the peak-force error images, we see that the circularly shaped particles with ~20-nm height and ~120-nm diameter (Fig. 8E) reside on top of small high-rise regions. The observed composite of a particle on a flat high-rise region resembles the organization of an isolated vesicle on a flat bilayer patch⁸⁹. The later structure represents an intermediate state during the vesicle fusion process to form a flat bilayer⁸⁹. Therefore, our observation of globular particles coexisting with high-rise regions strongly supports that in the presence of fusion promoters (including the peptide itself and the ionic lipids), the extracted lipids by PrP106-126 can aggregate and fuse onto an existing bilayer surface to form the observed high-rise regions. It is noteworthy that a broad spectrum of amphipathic peptides has been shown to exhibit fusogenic properties^{90–92}. Similarly, PrP106-126 was found to augment membrane fusion^{15, 93}. It is plausible that the extracted lipids by PrP106-126 can aggregate with the peptide to form micellar structures. Due to the fusion tendency facilitated by PrP106-126, Chol, anionic lipids, and/or acidic pH, the dispersed lipid/peptide micelles can fuse onto existing bilayer surfaces to form the observed liposome-like particles and high-rise regions.

AFM-based force spectroscopy

To further explore the impact of PrP106-126 on mechanical properties of POPC/Chol bilayers, we use AFM-based force spectroscopy. After obtaining multiple force curves at each peptide concentration, we use a modified Hertz model to determine the bilayer Young's modulus E and the bilayer surface position s_0 . A water layer thickness d_{WL} of 2 nm is used for the data analysis. If we decrease d_{WL} to 1 nm^{59, 62}, the E value increases by ~20%; however, the bilayer surface position s_0 remains virtually the same. The insensitivity of s_0 to the choice of d_{WL} is likely related to the fact that s_0 is primarily determined by the position where the force vanishes near the bilayer surface. Assuming a water layer thickness d_{WL} of 1 nm, the bilayer thickness becomes $h=s_0-d_{WL}=5.5$ nm. This value is larger than the reported bilayer thickness of POPC⁷⁷. Using neutron and X-ray scattering experiments, Ku erka et al.

found that the overall bilayer thickness of a POPC bilayer is 4 nm⁷⁷. Moreover, the authors showed that the terminal trimethyl groups of POPC in the headgroup region are separated by 4.2 nm in opposing leaflets⁷⁷. This value agrees well with our bilayer thickness of 4.5 nm when assuming a water layer thickness of 2 nm.

Oriented multilamellar stacks deposited on solid substrates have been broadly used to characterize bilayer physical properties⁹⁴. The lamellar repeat spacing at full hydration is 6.4 nm for oriented POPC stacks⁹⁵. Similar repeat spacing for POPC multilamellar vesicles has been reported⁹⁶. The lamellar repeat spacing is contributed by the bilayer thickness and the thickness of an inter-bilayer water layer. Since our determined value of s_0 (6.5 nm) is virtually the same as the repeat spacing of multilamellar stacks, the water layer thickness d_{WL} in our single bilayer system must be similar to the thickness of the inter-bilayer water layer in fully hydrated multilamellar stacks. This observation supports the use of $d_{WL}=2$ nm instead of 1 nm that was used by Hugel and coworkers^{59, 62}.

Our force spectroscopy data show that the addition of PrP106-126 to POPC/Chol bilayers results in noticeable decreases of the bilayer Young's modulus E and the bilayer puncture force F_p . Increasing Chol content does not negate the effect of the peptide. Bilayer mechanical parameters including the area compressibility modulus, bending modulus, and line tension are proportional to the Young's modulus E ^{60, 97}. Therefore, our force spectroscopy data indicate that PrP106-126 affects bilayer mechanical properties. The reduced mechanical integrity caused by PrP106-126 is shared by many pore-forming AMPs, such as melittin⁹⁸, magainin²⁹⁹, and alamethicin^{100–102}. A recent study using AFM imaging showed that many AMPs function by reducing the edge stability of lipid membranes, resulting in porous defects and worm-like structures at elevated concentrations²⁹. Formation of pores driven by reduced membrane line tension in the presence of amphipathic peptides has also been widely suggested¹⁰³. Our observation of porous defects (Fig. 2) is consistent with the force spectroscopy data that PrP106-126 produces membrane defects by reducing the bilayer Young's modulus E and the related line tension.

Raman microspectroscopy

Changes of Raman spectral features are useful to reveal alterations of lipid vibrational dynamics induced by extrinsic polypeptides and environmental conditions^{104–105}. Using Raman microspectroscopy, we find that PrP106-126 confers distinct impacts on atomic vibrational dynamics of POPC and POPE dispersions. Specifically, PrP106-126 disorders the intra-chain C-C conformation of POPC acyl chains, while a negligible impact is exerted on POPE acyl chains. Moreover, PrP106-126 increases the inter-chain interaction of POPE acyl chains, while a minimal impact is found on the inter-chain packing of POPC acyl chains. The disparate effects are likely caused by different interaction modes between the peptide and the lipid matrix. Indeed, a thermal calorimetry study showed that modulation of the phase transition behavior induced by PrP106-126 was different for phosphatidylcholine and phosphatidylethanolamine bilayers¹⁰⁶.

Compared to POPC, the headgroup of POPE is smaller due to the absence of the trimethyl groups¹⁰⁷. The resulting POPE bilayer contains a larger solvent-exposed pocket in the headgroup region to incorporate surface-associated peptides. In addition, peptides can be

stabilized at the headgroup region by forming hydrogen bonds with the terminal ammonium of POPE lipids¹⁰⁸. Both the larger empty pocket and the hydrogen bonding capability of POPE will position PrP106-126 at a further distance away from the hydrophobic midplane. Based on the insignificant impact on the intra-chain C-C conformation of POPE acyl chains, it is plausible that the peptide does not insert deep into the hydrophobic core. On the other hand, PrP106-126 increases the inter-chain interaction of POPE acyl chains. Therefore, a large segment of the hydrophobic C-terminus of PrP106-126 likely resides right underneath the glycerol-carbonyl backbone (i.e., the peptide lies almost parallel to the bilayer surface). Lateral pressure profile calculation indicates that the backbone region contains the largest negative pressure^{109–110}. Peptide insertion into this region will relieve the negative pressure and may cause an enhanced chain-chain interaction.

The insertion depth of the peptide likely becomes larger in POPC bilayers due to the bulky trimethyl groups in the headgroup region. Interaction with the inserted hydrophobic C-terminus of PrP106-126 causes a disordering effect on the C-C conformation of POPC acyl chains. This explains the enhanced intensity ratio of the *gauche* and *trans* bands observed in the SOM region of POPC dispersions (Fig. 5). Since we do not see noticeable changes of the stretching bands of C-H in methylene groups, the inter-chain interaction of POPC acyl chains is not perturbed by peptide insertion. Our observation of lipid-dependent Raman spectral perturbation caused by PrP106-126 is in line with reports showing that cone-shaped phosphatidylethanolamine lipids are more susceptible to structural reorganization induced by various peptides^{111–114}.

Electron paramagnetic resonance

Spin-labeling EPR spectroscopy is a sensitive technique to probe conformational order of model lipid membranes. Compared to isotropic vesicles, orientation-amenable bicelles offer an additional advantage. By magnetically aligning bicelles, the sensitivity of EPR signal to lipid orientation and order is enhanced^{52, 54, 115–116}. Effects of administrated peptides on lipid orientational order can be assessed by analysis of spectral line shapes. A parameter that has been used to describe the orientational order of lipid acyl chains in bicelles is S_{20} , which is obtained by globally simulating experimental EPR spectra. A reduction in S_{20} reflects a loss of the orientational order of lipid acyl chains. Moreover, the extent of the reduction of S_{20} at different P/L ratios is correlated with the action mode of the peptide. For example, peptides forming barrel-stave pores have a modest impact on the orientational order of lipid acyl chains, whereas peptides forming toroidal pores (or having a detergent-like effect) can cause a strong disordering on lipid chain orientational order⁵². Based on the outlined criteria, the authors proceeded to evaluate the mechanism of membrane disruption caused by AMPs that had not been well-characterized⁵².

Our EPR spectroscopy measurements of magnetically aligned bicelles show that spectral line shapes are progressively altered as the concentration of PrP106-126 increases. Using spectral simulations, we quantify the effect of PrP106-126 on lipid chain orientational order by determining the parameter S_{20} at different P/L ratios. Note that the orientational order parameter S_{20} presented here is different from the order parameter determined from nuclear magnetic resonance (NMR) measurements^{117–118}. Our determined S_{20} for pure bicelles is

close to the reported ones on similar systems^{52, 54}. The addition of PrP106-126 at low P/L ratios (i.e. 1/300 and 1/200) causes a mild decrease of S_{20} . The modest decrease is likely caused by initial peptide binding in a monomeric state with the N-terminus anchored at the bilayer-water interface and the C-terminus inserted into the hydrophobic core. Interactions with partially inserted monomeric peptides perturb the packing property of neighboring lipid chains, resulting in a decreased S_{20} . Despite the perturbation, the overall bilayer organization is maintained. The proposed mechanism of PrP106-126 at low peptide contents is similar to that of alamethicin, which decreases S_{20} by modulating lipid chain packing in the vicinity of multimeric channels^{52, 54}.

Increasing the content of PrP106-126 to P/L 1/75 results in a marked decrease of S_{20} that almost reaches the isotropic limit of $S_{20}=0$. Based on the similarity of the vanishing S_{20} caused by PrP106-126 and magainin 2, we speculate that toroidal-shaped defects lined by PrP106-126 monomers and lipid molecules are formed at P/L 1/75. Formation of toroidal defects in bicelles is supported by our AFM imaging data (Fig. 2). Since S_{20} almost vanishes at P/L 1/75, the number and/or size of the proposed toroidal defects must be large. An alternative picture is that PrP106-126 extracts bicellar lipids to form isotropic peptide/lipid micelles. The lipid extraction (or micellization) mechanism is supported by the large sizes of porous defects observed in AFM images. Similar micellization mechanism of AMPs that can cause positive curvature has been described^{81, 119}. Micellization of lipid membranes has also been proposed for oligomers formed by PrP106-126³⁰. The two mechanisms proposed here likely coexist/overlap such that the mechanism of forming toroidal defects dominates at low peptide contents (but high enough to induce defects), while the micellization mechanism prevails at high peptide contents²⁹.

Conclusions

In this study, we use several experimental approaches to interrogate the effects of monomeric PrP106-126 on physical properties of model lipid membranes. Specifically, we use solution AFM to visualize peptide-induced porous defects in POPC planar bilayers. The addition of Chol up to 50 mol% does not impede defect development. In parallel with bilayer topographic modulation, our AFM-based force spectroscopy measurements reveal that PrP106-126 reduces the bilayer Young's modulus E and the bilayer puncture force F_p . Impairment of the bilayer mechanical integrity supports the notion that PrP106-126 induces porous defects by modulating membrane physical parameters such as the line tension. Raman microspectroscopy is used to explore atomic-level interactions between PrP106-126 and lipid dispersions. We find that the effect of PrP106-126 on lipid vibrational dynamics is dependent on membrane physical properties. For cylindrical POPC molecules, the addition of PrP106-126 results in an enhanced intra-chain conformational disorder, while the inter-chain interaction is not altered; for cone-shaped POPE molecules, the addition of PrP106-126 increases the inter-chain interaction, while the intra-chain conformational order remains similar. We explain the observed differences by considering different modes of peptide insertion into POPC and POPE bilayers. Finally, EPR spectroscopy on magnetically aligned bicelles is used to investigate the impact of PrP106-126 on the orientational order of lipid acyl chains. We find that the addition of PrP106-126 progressively decreases the orientational order parameter S_{20} . By comparing to two reported peptides (i.e. alamethicin

and magainin 2), our EPR data suggest that PrP106-126 disorders lipid orientational order moderately at low P/L ratios, while higher P/L ratios result in the formation of toroidal defects and lipid/peptide micelles. Together, our experimental data support the hypothesis that the action mode of the amphipathic peptide PrP106-126 resembles that of many membrane-active AMPs. This conclusion is in line with the proposition that the preamyloid state of amyloidogenic peptides shares common physical principles with AMPs in interacting with lipid membranes¹²⁰.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Prusiner SB. Prions. *Proc Natl Acad Sci U S A*. 1998; 95:13363–13383. [PubMed: 9811807]
2. Forloni G, Angeretti N, Chiesa R, Monzani E, Salmona M, Bugiani O, Tagliavini F. Neurotoxicity of a prion protein-fragment. *Nature*. 1993; 362:543–546. [PubMed: 8464494]
3. Salmona M, Malesani P, De Gioia L, Gorla S, Bruschi M, Molinari A, Della Vedova F, Pedrotti B, Marrari MA, Awan T, et al. Molecular determinants of the physicochemical properties of a critical prion protein region comprising residues 106-126. *Biochem J*. 1999; 342:207–214. [PubMed: 10432318]
4. Ettaiche M, Pichot R, Vincent JP, Chabry J. In vivo cytotoxicity of the prion protein fragment 106-126. *J Biol Chem*. 2000; 275:36487–36490. [PubMed: 11007766]
5. Deli MA, Sakaguchi S, Nakaoke R, Abraham CS, Takahata H, Kopacek J, Shigematsu K, Katamine S, Niwa M. Prp fragment 106-126 is toxic to cerebral endothelial cells expressing prpc. *NeuroReport*. 2000; 11:3931–3936. [PubMed: 11117517]
6. Cooper I, Malina KCK, Cagnotto A, Bazzoni G, Salmona M, Teichberg VI. Interactions of the prion peptide (prp 106-126) with brain capillary endothelial cells: Coordinated cell killing and remodeling of intercellular junctions. *J Neurochem*. 2011; 116:467–475. [PubMed: 20804519]
7. Kunz B, Sandmeier E, Christen P. Neurotoxicity of prion peptide 106-126 not confirmed. *FEBS Lett*. 1999; 458:65–68. [PubMed: 10518935]
8. Florio T, Thellung S, Amico C, Robello M, Salmona M, Bugiani O, Tagliavini F, Forloni G, Schettini G. Prion protein fragment 106-126 induces apoptotic cell death and impairment of l-type voltage-sensitive calcium channel activity in the gh3 cell line. *J Neurosci Res*. 1998; 54:341–352. [PubMed: 9819139]
9. Thellung S, Florio T, Villa V, Corsaro A, Arena S, Amico C, Robello M, Salmona M, Forloni G, Bugiani O, et al. Apoptotic cell death and impairment of l-type voltage-sensitive calcium channel activity in rat cerebellar granule cells treated with the prion protein fragment 106-126. *Neurobiol Dis*. 2000; 7:299–309. [PubMed: 10964602]
10. O'Donovan CN, Tobin D, Cotter TG. Prion protein fragment prp-(106-126) induces apoptosis via mitochondrial disruption in human neuronal sh-sy5y cells. *J Biol Chem*. 2001; 276:43516–43523. [PubMed: 11533027]
11. Rymer DL, Good TA. The role of prion peptide structure and aggregation in toxicity and membrane binding. *J Neurochem*. 2000; 75:2536–2545. [PubMed: 11080207]

12. Florio T, Paludi D, Villa V, Principe DR, Corsaro A, Millo E, Damonte G, D'Arrigo C, Russo C, Schettini G, et al. Contribution of two conserved glycine residues to fibrillogenesis of the 106-126 prion protein fragment. Evidence that a soluble variant of the 106-126 peptide is neurotoxic. *J Neurochem*. 2003; 85:62–72. [PubMed: 12641727]
13. Carimalo J, Cronier S, Petit G, Peyrin JM, Boukhtouche F, Arbez N, Lemaigre-Dubreuil Y, Brugg B, Miquel MC. Activation of the jnk-c-jun pathway during the early phase of neuronal apoptosis induced by prp106-126 and prion infection. *Eur J Neurosci*. 2005; 21:2311–2319. [PubMed: 15932590]
14. Fioriti L, Quaglio E, Massignan T, Colombo L, Stewart RS, Salmona M, Harris DA, Forloni G, Chiesa R. The neurotoxicity of prion protein (prp) peptide 106-126 is independent of the expression level of prp and is not mediated by abnormal prp species. *Mol Cell Neurosci*. 2005; 28:165–176. [PubMed: 15607951]
15. Dupiereux I, Zorzi W, Lins L, Brasseur R, Colson P, Heinen E, Elmoualij B. Interaction of the 106-126 prion peptide with lipid membranes and potential implication for neurotoxicity. *Biochem Biophys Res Commun*. 2005; 331:894–901. [PubMed: 15882962]
16. Dupiereux I, Zorzi W, Rachidi W, Zorzi D, Pierard O, Lhereux B, Heinen E, Elmoualij B. Study on the toxic mechanism of prion protein peptide 106-126 in neuronal and non neuronal cells. *J Neurosci Res*. 2006; 84:637–646. [PubMed: 16786576]
17. Pietri M, Caprini A, Mouillet-Richard S, Pradines E, Ermonval M, Grassi J, Kellermann O, Schneider B. Overstimulation of prpc signaling pathways by prion peptide 106-126 causes oxidative injury of bioaminergic neuronal cells. *J Biol Chem*. 2006; 281:28470–28479. [PubMed: 16864581]
18. Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell*. 1987; 51:229–240. [PubMed: 2444340]
19. Vey M, Pilkuhn S, Wille H, Nixon R, Dearmond SJ, Smart EJ, Anderson RGW, Taraboulos A, Prusiner SB. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proc Natl Acad Sci U S A*. 1996; 93:14945–14949. [PubMed: 8962161]
20. Naslavsky N, Shmeeda H, Friedlander G, Yanai A, Futerman AH, Barenholz Y, Taraboulos A. Sphingolipid depletion increases formation of the scrapie prion protein in neuroblastoma cells infected with prions. *J Biol Chem*. 1999; 274:20763–20771. [PubMed: 10409615]
21. Pinheiro TJJ. The role of rafts in the fibrillization and aggregation of prions. *Chem Phys Lipids*. 2006; 141:66–71. [PubMed: 16647049]
22. Butterfield SM, Lashuel HA. Amyloidogenic protein membrane interactions: Mechanistic insight from model systems. *Angew Chem, Int Ed*. 2010; 49:5628–5654.
23. Botto L, Cunati D, Coco S, Sesana S, Bulbarelli A, Biasini E, Colombo L, Negro A, Chiesa R, Masserini M, et al. Role of lipid rafts and gm1 in the segregation and processing of prion protein. *PLoS One*. 2014; 9:e98344. [PubMed: 24859148]
24. Ilc G, Giachin G, Jaremko M, Jaremko L, Benetti F, Plavec J, Zhukov I, Legname G. Nmr structure of the human prion protein with the pathological q212p mutation reveals unique structural features. *PLoS One*. 2010; 5
25. Hancock REW, Lehrer R. Cationic peptides: A new source of antibiotics. *Trends Biotechnol*. 1998; 16:82–88. [PubMed: 9487736]
26. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature*. 2002; 415:389–395. [PubMed: 11807545]
27. De Gioia L, Selvaggini C, Ghibaudi E, Diomede L, Bugiani O, Forloni G, Tagliavini F, Salmona M. Conformational polymorphism of the amyloidogenic and neurotoxic peptide homologous to residues-106-126 of the prion protein. *J Biol Chem*. 1994; 269:7859–7862. [PubMed: 7907586]
28. Villa A, Mark AE, Saracino GAA, Cosentino U, Pitea D, Moro G, Salmona M. Conformational polymorphism of the prp106-126 peptide in different environments: A molecular dynamics study. *J Phys Chem B*. 2006; 110:1423–1428. [PubMed: 16471693]
29. Henderson JM, Waring AJ, Separovic F, Lee KYC. Antimicrobial peptides share a common interaction driven by membrane line tension reduction. *Biophys J*. 2016; 101:2176–2189.

30. Walsh P, Vanderlee G, Yau J, Campeau J, Sim VL, Yip CM, Sharpe S. The mechanism of membrane disruption by cytotoxic amyloid oligomers formed by prion protein(106-126) is dependent on bilayer composition. *J Biol Chem.* 2014; 289:10419–10430. [PubMed: 24554723]
31. Walsh P, Yau J, Simonetti K, Sharpe S. Morphology and secondary structure of stable beta-oligomers formed by amyloid peptide prp(106-126). *Biochemistry.* 2009; 48:5779–5781. [PubMed: 19476383]
32. Zhong J, Zheng WF, Huang LX, Hong YK, Wang LJ, Qiu Y, Sha YL. Prp106-126 amide causes the semi-penetrated poration in the supported lipid bilayers. *Biochim Biophys Acta, Biomembr.* 2007; 1768:1420–1429.
33. Zheng WF, Wang LJ, Hong YK, Sha YL. Prp106-126 peptide disrupts lipid membranes: Influence of c-terminal amidation. *Biochem Biophys Res Commun.* 2009; 379:298–303. [PubMed: 19103159]
34. Zhong J, Yang CH, Zheng WF, Huang LX, Hong YK, Wang LJ, Sha YL. Effects of lipid composition and phase on the membrane interaction of the prion peptide 106-126 amide. *Biophys J.* 2009; 96:4610–4621. [PubMed: 19486683]
35. Zhong J, Yang CH, Zheng WF, Huang LX, Hong YK, Wang LJ, Sha YL. The role of calcium ions in the interactions of prp106-126 amide with model membranes. *Colloids Surf, B.* 2010; 77:40–46.
36. Henriques ST, Pattenden LK, Aguilar MI, Castanho MARB. Prp(106-126) does not interact with membranes under physiological conditions. *Biophys J.* 2008; 95:1877–1889. [PubMed: 18469080]
37. Heegaard PMH, Pedersen HG, Flink J, Boas U. Amyloid aggregates of the prion peptide prp106-126 are destabilised by oxidation and by the action of dendrimers. *FEBS Lett.* 2004; 577:127–133. [PubMed: 15527773]
38. Bergstrom AL, Cordes H, Zsurgner N, Heegaard PMH, Laursen H, Chabry J. Amidation and structure relaxation abolish the neurotoxicity of the prion peptide prp106-126 in vivo and in vitro. *J Biol Chem.* 2005; 280:23114–23121. [PubMed: 15824101]
39. Sun Y, Hung WC, Lee MT, Huang HW. Membrane-mediated amyloid formation of prp 106-126: A kinetic study. *Biochim Biophys Acta, Biomembr.* 2015; 1848:2422–2429.
40. Khadka NK, Cheng XL, Ho CS, Katsaras J, Pan JJ. Interactions of the anticancer drug tamoxifen with lipid membranes. *Biophys J.* 2015; 108:2492–2501. [PubMed: 25992727]
41. Khadka NK, Ho CS, Pan JJ. Macroscopic and nanoscopic heterogeneous structures in a three-component lipid bilayer mixtures determined by atomic force microscopy. *Langmuir.* 2015; 31:12417–12425. [PubMed: 26506226]
42. Ho CS, Khadka NK, Pan JJ. Sub-ten-nanometer heterogeneity of solid supported lipid membranes determined by solution atomic force microscopy. *Biochim Biophys Acta, Biomembr.* 2016; 1858:181–188.
43. Ho CS, Khadka NK, She FY, Cai JF, Pan JJ. Polyglutamine aggregates impair lipid membrane integrity and enhance lipid membrane rigidity. *Biochim Biophys Acta, Biomembr.* 2016; 1858:661–670.
44. Ho CS, Khadka NK, She FY, Cai JF, Pan JJ. Influenza m2 transmembrane domain senses membrane heterogeneity and enhances membrane curvature. *Langmuir.* 2016; 32:6730–6738. [PubMed: 27285399]
45. Pan JJ, Khadka NK. Kinetic defects induced by melittin in model lipid membranes: A solution atomic force microscopy study. *J Phys Chem B.* 2016; 120:4625–4634. [PubMed: 27167473]
46. Khadka NK, Teng P, Cai J, Pan J. Modulation of lipid membrane structural and mechanical properties by a peptidomimetic derived from reduced amide scaffold. *Biochim Biophys Acta, Biomembr.* 2017; 1859:734–744.
47. Reviakine I, Brisson A. Formation of supported phospholipid bilayers from unilamellar vesicles investigated by atomic force microscopy. *Langmuir.* 2000; 16:1806–1815.
48. Leonenko ZV, Carnini A, Cramb DT. Supported planar bilayer formation by vesicle fusion: The interaction of phospholipid vesicles with surfaces and the effect of gramicidin on bilayer properties using atomic force microscopy. *Biochim Biophys Acta, Biomembr.* 2000; 1509:131–147.
49. Wojdyr M. Fityk: A general-purpose peak fitting program. *J Appl Crystallogr.* 2010; 43:1126–1128.

50. Cardon TB, Tiburu EK, Lorigan GA. Magnetically aligned phospholipid bilayers in weak magnetic fields: Optimization, mechanism, and advantages for x-band epr studies. *J Magn Reson.* 2003; 161:77–90. [PubMed: 12660114]
51. Mangels ML, Cardon TB, Harper AC, Howard KP, Lorigan GA. Spectroscopic characterization of spin-labeled magnetically oriented phospholipid bilayers by epr spectroscopy. *J Am Chem Soc.* 2000; 122:7052–7058.
52. Bortolus M, Dalzini A, Toniolo C, Hahm KS, Maniero AL. Interaction of hydrophobic and amphipathic antimicrobial peptides with lipid bicelles. *J Pept Sci.* 2014; 20:517–525. [PubMed: 24863176]
53. Budil DE, Lee S, Saxena S, Freed JH. Nonlinear-least-squares analysis of slow-motion epr spectra in one and two dimensions using a modified levenberg-marquardt algorithm. *J Magn Reson Ser A.* 1996; 120:155–189.
54. Bortolus M, De Zotti M, Formaggio F, Maniero AL. Alamethicin in bicelles: Orientation, aggregation, and bilayer modification as a function of peptide concentration. *Biochim Biophys Acta, Biomembr.* 2013; 1828:2620–2627.
55. Nusair NA, Lorigan GA. Investigating the structural and dynamic properties of n-doxylstearic acid in magnetically-aligned phospholipid bilayers by x-band epr spectroscopy. *Chem Phys Lipids.* 2005; 133:151–164. [PubMed: 15642584]
56. Inbaraj JJ, Cardon TB, Laryukhin M, Grosser SM, Lorigan GA. Determining the topology of integral membrane peptides using epr spectroscopy. *J Am Chem Soc.* 2006; 128:9549–9554. [PubMed: 16848493]
57. Jeong JK, Seol JW, Moon MH, Seo JS, Lee YJ, Kim JS, Park SY. Cellular cholesterol enrichment prevents prion peptide-induced neuron cell damages. *Biochem Biophys Res Commun.* 2010; 401:516–520. [PubMed: 20875400]
58. Dimitriadis EK, Horkay F, Maresca J, Kachar B, Chadwick RS. Determination of elastic moduli of thin layers of soft material using the atomic force microscope. *Biophys J.* 2002; 82:2798–2810. [PubMed: 11964265]
59. Stetter FWS, Hugel T. The nanomechanical properties of lipid membranes are significantly influenced by the presence of ethanol. *Biophys J.* 2013; 104:1049–1055. [PubMed: 23473487]
60. Picas L, Rico F, Scheuring S. Direct measurement of the mechanical properties of lipid phases in supported bilayers. *Biophys J.* 2012; 102:L1–L3.
61. Wang X, Sanderson RN, Ragan R. Evaluation of young's modulus of tethered 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine membranes using atomic force spectroscopy. *J Phys Chem C.* 2014; 118:29301–29309.
62. Stetter FWS, Hyun SH, Brander S, Urban JM, Thompson DH, Hugel T. Nanomechanical characterization of lipid bilayers with afm-based methods. *Polymer.* 2016; 102:326–332.
63. Kell GS. Density, thermal expansivity, and compressibility of liquid water from 0 degrees to 150 degrees c - correlations and tables for atmospheric-pressure and saturation reviewed and expressed on 1968 temperature scale. *J Chem Eng Data.* 1975; 20:97–105.
64. Henriksen J, Rowat AC, Brief E, Hsueh YW, Thewalt JL, Zuckermann MJ, Ipsen JH. Universal behavior of membranes with sterols. *Biophys J.* 2006; 90:1639–1649. [PubMed: 16326903]
65. Pan JJ, Mills TT, Tristram-Nagle S, Nagle JF. Cholesterol perturbs lipid bilayers nonuniversally. *Phys Rev Lett.* 2008; 100:198103. [PubMed: 18518492]
66. Pan JJ, Tristram-Nagle S, Nagle JF. Effect of cholesterol on structural and mechanical properties of membranes depends on lipid chain saturation. *Phys Rev E.* 2009; 80:021931.
67. Lippert JL, Peticolas WL. Laser raman investigation of effect of cholesterol on conformational changes in dipalmitoyl lecithin multilayers - (gel-liquid crystal transition/noncooperative/transition temperature). *Proc Natl Acad Sci U S A.* 1971; 68:1572–1576. [PubMed: 5283948]
68. Gaber BP, Peticolas WL. On the quantitative interpretation of biomembrane structure by raman spectroscopy. *Biochim Biophys Acta.* 1977; 465:260–274. [PubMed: 16250339]
69. Spiker RC, Levin IW. Raman spectra and vibrational assignments for dipalmitoyl phosphatidylcholine and structurally related molecules. *Biochim Biophys Acta.* 1975; 388:361–373. [PubMed: 1137716]

70. Mushayakarara E, Levin IW. Determination of acyl chain conformation at the lipid interface region - raman-spectroscopic study of the carbonyl stretching mode region of dipalmitoyl phosphatidylcholine and structurally related molecules. *J Phys Chem.* 1982; 86:2324–2327.
71. Mushayakarara E, Albon N, Levin IW. Effect of water on the molecular-structure of a phosphatidylcholine hydrate - raman-spectroscopic analysis of the phosphate, carbonyl and carbon-hydrogen stretching mode regions of 1,2-dipalmitoylphosphatidylcholine dihydrate. *Biochim Biophys Acta.* 1982; 686:153–159. [PubMed: 6896285]
72. Bush SF, Adams RG, Levin IW. Structural reorganizations in lipid bilayer systems - effect of hydration and sterol addition on raman-spectra of dipalmitoylphosphatidylcholine multilayers. *Biochemistry.* 1980; 19:4429–4436. [PubMed: 6893277]
73. Fox RO, Richards FM. A voltage-gated ion channel model inferred from the crystal-structure of alamethicin at 1.5-Å resolution. *Nature.* 1982; 300:325–330. [PubMed: 6292726]
74. Matsuzaki K. Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochim Biophys Acta, Rev Biomembr.* 1998; 1376:391–400.
75. Papo N, Shai Y. Exploring peptide membrane interaction using surface plasmon resonance: Differentiation between pore formation versus membrane disruption by lytic peptides. *Biochemistry.* 2003; 42:458–466. [PubMed: 12525173]
76. Li C, Salditt T. Structure of magainin and alamethicin in model membranes studied by x-ray reflectivity. *Biophys J.* 2006; 91:3285–3300. [PubMed: 16920839]
77. Ku erka N, Nieh MP, Katsaras J. Fluid phase lipid areas and bilayer thicknesses of commonly used phosphatidylcholines as a function of temperature. *Biochim Biophys Acta, Biomembr.* 2011; 1808:2761–2771.
78. Butt HJ. Measuring electrostatic, van der waals, and hydration forces in electrolyte solutions with an atomic force microscope. *Biophys J.* 1991; 60:1438–1444. [PubMed: 19431815]
79. Lin MX, Mirzabekov T, Kagan BL. Channel formation by a neurotoxic prion protein fragment. *J Biol Chem.* 1997; 272:44–47. [PubMed: 8995224]
80. Kourie JJ, Culverson A. Prion peptide fragment prp[106-126] forms distinct cation channel types. *J Neurosci Res.* 2000; 62:120–133. [PubMed: 11002294]
81. Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim Biophys Acta, Biomembr.* 1999; 1462:55–70.
82. Rakowska PD, Jiang HB, Ray S, Pyne A, Lamarre B, Carr M, Judge PJ, Ravi J, Gerling UIM, Koksche B, et al. Nanoscale imaging reveals laterally expanding antimicrobial pores in lipid bilayers. *Proc Natl Acad Sci U S A.* 2013; 110:8918–8923. [PubMed: 23671080]
83. Connor J, Yatvin MB, Huang L. Ph-sensitive liposomes - acid-induced liposome fusion. *Proc Natl Acad Sci U S A.* 1984; 81:1715–1718. [PubMed: 6584905]
84. Driessen AJM, Hoekstra D, Scherphof G, Kalicharan RD, Wilschut J. Low ph-induced fusion of liposomes with membrane-vesicles derived from bacillus-subtilis. *J Biol Chem.* 1985; 260:880–887. [PubMed: 3968070]
85. Oku N, Shibamoto S, Ito F, Gondo H, Nango M. Low ph induced membrane-fusion of lipid vesicles containing proton-sensitive polymer. *Biochemistry.* 1987; 26:8145–8150. [PubMed: 3442648]
86. Bailey AL, Monck MA, Cullis PR. Ph-induced destabilization of lipid bilayers by a lipopeptide derived from influenza hemagglutinin. *Biochim Biophys Acta, Biomembr.* 1997; 1324:232–244.
87. Yang ST, Kreutzberger AJB, Lee J, Kiessling V, Tamm LK. The role of cholesterol in membrane fusion. *Chem Phys Lipids.* 2016; 199:136–143. [PubMed: 27179407]
88. Fujii G, Selsted ME, Eisenberg D. Defensins promote fusion and lysis of negatively charged membranes. *Protein Sci.* 1993; 2:1301–1312. [PubMed: 8401215]
89. Tokumasu F, Jin AJ, Feigenson GW, Dvorak JA. Atomic force microscopy of nanometric liposome adsorption and nanoscopic membrane domain formation. *Ultramicroscopy.* 2003; 97:217–227. [PubMed: 12801674]
90. Parente RA, Nir S, Szoka FC. Ph-dependent fusion of phosphatidylcholine small vesicles - induction by a synthetic amphipathic peptide. *J Biol Chem.* 1988; 263:4724–4730. [PubMed: 2450874]

91. Murata M, Takahashi S, Kagiwada S, Suzuki A, Ohnishi S. Ph-dependent membrane-fusion and vesiculation of phospholipid large unilamellar vesicles induced by amphiphilic anionic and cationic peptides. *Biochemistry*. 1992; 31:1986–1992. [PubMed: 1536841]
92. Martens S, McMahon HT. Mechanisms of membrane fusion: Disparate players and common principles. *Nat Rev Mol Cell Biol*. 2008; 9:543–556. [PubMed: 18496517]
93. Kurganov B, Doh M, Arispe N. Aggregation of liposomes induced by the toxic peptides alzheimer's a beta s, human amylin and prion (106-126): Facilitation by membrane-bound g(m1) ganglioside. *Peptides*. 2004; 25:217–232. [PubMed: 15063003]
94. Nagle JF, Tristram-Nagle S. Structure of lipid bilayers. *Biochim Biophys Acta, Rev Biomembr*. 2000; 1469:159–195.
95. Kucerka N, Tristram-Nagle S, Nagle JF. Structure of fully hydrated fluid phase lipid bilayers with monounsaturated chains. *J Membr Biol*. 2005; 208:193–202. [PubMed: 16604469]
96. Pabst G, Hodzic A, Strancar J, Danner S, Rappolt M, Laggner P. Rigidification of neutral lipid bilayers in the presence of salts. *Biophys J*. 2007; 93:2688–2696. [PubMed: 17586572]
97. Rawicz W, Olbrich KC, McIntosh T, Needham D, Evans E. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys J*. 2000; 79:328–339. [PubMed: 10866959]
98. Pott T, Gerbeaud C, Barbier N, Meleard P. Melittin modifies bending elasticity in an unexpected way. *Chem Phys Lipids*. 2015; 185:99–108. [PubMed: 24875586]
99. Bouvrais H, Meleard P, Pott T, Jensen KJ, Brask J, Ipsen JH. Softening of popc membranes by magainin. *Biophys Chem*. 2008; 137:7–12. [PubMed: 18602207]
100. Vitkova V, Meleard P, Pott T, Bivas I. Alamethicin influence on the membrane bending elasticity. *Eur Biophys J*. 2006; 35:281–286. [PubMed: 16211403]
101. Pabst G, Danner S, Podgornik R, Katsaras J. Entropy-driven softening of fluid lipid bilayers by alamethicin. *Langmuir*. 2007; 23:11705–11711. [PubMed: 17939689]
102. Pan JJ, Tieleman DP, Nagle JF, Kucerka N, Tristram-Nagle S. Alamethicin in lipid bilayers: Combined use of x-ray scattering and md simulations. *Biochim Biophys Acta, Biomembr*. 2009; 1788:1387–1397.
103. Fuertes G, Gimenez D, Esteban-Martin S, Sanchez-Munoz OL, Salgado J. A lipocentric view of peptide-induced pores. *Eur Biophys J*. 2011; 40:399–415. [PubMed: 21442255]
104. Levin IW, Lavialle F, Mollay C. Comparative effects of melittin and its hydrophobic and hydrophilic fragments on bilayer organization by raman-spectroscopy. *Biophys J*. 1982; 37:339–349. [PubMed: 7055626]
105. Rusciano G, Pesce G, Zito G, Sasso A, Gaglione R, Del Giudice R, Piccoli R, Monti DM, Arciello A. Insights into the interaction of the n-terminal amyloidogenic polypeptide of apoa-i with model cellular membranes. *Biochim Biophys Acta, Gen Subj*. 2016; 1860:795–801.
106. Grasso D, Milardi D, La Rosa C, Rizzarelli E. Dsc study of the interaction of the prion peptide prp106-126 with artificial membranes. *New J Chem*. 2001; 25:1543–1548.
107. Kucerka N, van Oosten B, Pan JJ, Heberle FA, Harroun TA, Katsaras J. Molecular structures of fluid phosphatidylethanolamine bilayers obtained from simulation-to-experiment comparisons and experimental scattering density profiles. *J Phys Chem B*. 2015; 119:1947–1956. [PubMed: 25436970]
108. Bazzi MD, Youakim A, Nelsestuen GL. Importance of phosphatidylethanolamine for association of protein-kinase-c and other cytoplasmic proteins with membranes. *Biochemistry*. 1992; 31:1125–1134. [PubMed: 1734960]
109. Cantor RS. Lipid composition and the lateral pressure profile in bilayers. *Biophys J*. 1999; 76:2625–2639. [PubMed: 10233077]
110. Marsh D. Lateral pressure profile, spontaneous curvature frustration, and the incorporation and conformation of proteins in membranes. *Biophys J*. 2007; 93:3884–3899. [PubMed: 17704167]
111. Keller SL, Gruner SM, Gawrisch K. Small concentrations of alamethicin induce a cubic phase in bulk phosphatidylethanolamine mixtures. *Biochim Biophys Acta, Biomembr*. 1996; 1278:241–246.
112. Yang LH, Gordon VD, Mishra A, Sorn A, Purdy KR, Davis MA, Tew GN, Wong GCL. Synthetic antimicrobial, oligomers induce a composition-dependent topological transition in membranes. *J Am Chem Soc*. 2007; 129:12141–12147. [PubMed: 17880067]

113. Hickel A, Danner-Pongratz S, Amenitsch H, Degovics G, Rappolt M, Lohner K, Pabst G. Influence of antimicrobial peptides on the formation of nonlamellar lipid mesophases. *Biochim Biophys Acta, Biomembr.* 2008; 1778:2325–2333.
114. Koller D, Lohner K. The role of spontaneous lipid curvature in the interaction of interfacially active peptides with membranes. *Biochim Biophys Acta, Biomembr.* 2014; 1838:2250–2259.
115. McCaffrey JE, James ZM, Thomas DD. Optimization of bicelle lipid composition and temperature for epr spectroscopy of aligned membranes. *J Magn Reson.* 2015; 250:71–75. [PubMed: 25514061]
116. Cardon TB, Tiburu EK, Padmanabhan A, Howard KP, Lorigan GA. Magnetically aligned phospholipid bilayers at the parallel and perpendicular orientations for x-band spin-label epr studies. *J Am Chem Soc.* 2001; 123:2913–2914. [PubMed: 11456991]
117. Seelig A, Seelig J. Dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium magnetic-resonance. *Biochemistry.* 1974; 13:4839–4845. [PubMed: 4371820]
118. Binder H, Gawrisch K. Effect of unsaturated lipid chains on dimensions, molecular order and hydration of membranes. *J Phys Chem B.* 2001; 105:12378–12390.
119. Haney EF, Nathoo S, Vogel HJ, Prenner EJ. Induction of non-lamellar lipid phases by antimicrobial peptides: A potential link to mode of action. *Chem Phys Lipids.* 2010; 163:82–93. [PubMed: 19799887]
120. Last NB, Schlamadinger DE, Miranker AD. A common landscape for membrane-active peptides. *Protein Sci.* 2013; 22:870–882. [PubMed: 23649542]

Figure 1. The amphipathic structure of PrP106-126 derived from an NMR structure (PDB: 2KUN)²⁴. The peptide is shown in ball-stick and semitransparent surface representation. Hydrophilic residues (colored in green) are segregated at the N-terminus, whereas hydrophobic residues (colored in orange) are localized at the C-terminus.

Figure 2.

Solution AFM height images of (A) POPC, (B) POPC+30 mol% Chol, and (C) POPC+50 mol% Chol bilayers exposed to incremental concentrations of PrP106-126 at 0, 0.2, and 0.5 μM. The color bar in (A) shows the height scale used for all images. Scale bars are 2 μm. Semilogarithmic height probabilities of AFM images in A, B, and C are shown in D, E, and F, respectively.

Figure 3. Force spectroscopy data of (A) POPC, (B) POPC+30 mol% Chol, and (C) POPC+50 mol% Chol bilayers exposed to incremental concentrations of PrP106-126 at 0, 0.2, and 0.5 μM . Each panel contains a superposition of >300 force curves.

Figure 4.

Mechanical and structural parameters of POPC/Chol bilayers modified by the incremental addition of PrP106-126 from 0 to 0.5 μM . The bilayer Young's modulus E (A) and the bilayer surface position s_0 (B) are obtained from force data analysis using the modified Hertz model.

Figure 5.
Raman spectrum of POPC dispersions without and with PrP106-126 (24°C).

Figure 6.
Raman spectrum of POPE dispersions without and with PrP106-126 (30°C).

Figure 7.

Lipid order analysis of EPR spectra. (A) Experimental (solid lines) and simulated (dashed lines) EPR spectra of DMPC/DHPC bicelles containing the spin label 5-SASL. The bilayer normal of the bicelles is aligned parallel to the magnetic field at 308 K. (B) Plot of the orientational order parameter S_{20} as a function of the P/L ratio. For comparison, the reported values for alamethicin and magainin 2 are also displayed^{52, 54}.

Figure 8.

(A, B) Solution AFM height images of a POPC+10 mol% TOCL bilayer exposed to 0.5 μM PrP106-126 at pH 7.4. The two height images are obtained at the same location using two scans with different magnifications. (C, D) Peak-force error images obtained simultaneously with the height images shown in (A, B). (E) Height profiles along the dashed lines indicated in (A, B). Two types of lipid deposits are highlighted by green and blue arrows in (B, D). Scale bars are 1 μm .

Table 1

Mechanical and structural properties of POPC/Chol bilayers modulated by monomeric PrP106-126. The two parameters (E and s_0) are obtained by analyzing force data using the modified Hertz model. Based on the analysis of >300 force curves for each combination, E has an uncertainty of 4 MPa, and s_0 has an uncertainty of 0.2 nm. The uncertainty of F_p is 0.2 nN for control bilayers, and 0.1 nN for bilayers treated with 0.2 and 0.5 μM PrP106-126.

		0 μM	0.2 μM	0.5 μM
POPC	E (MPa)	59	55	53
	s_0 (nm)	6.5	6.6	6.6
	F_p (nN)	2.7	2.4	2.4
POPC+30% Chol	E (MPa)	62	54	52
	s_0 (nm)	7.0	7.2	7.3
	F_p (nN)	3.2	2.5	2.4
POPC+50% Chol	E (MPa)	85	55	53
	s_0 (nm)	7.5	7.6	7.6
	F_p (nN)	3.4	2.7	2.6