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Beyond NMR Spectra of Antimicrobial Peptides: Dynamical Images at Atomic Resolution and Functional Insights

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Abstract

There is a considerable current interest in understanding the function of antimicrobial peptides for the development of potent novel antibiotic compounds with a very high selectivity. Since their interaction with the cell membrane is the major driving force for their function, solid-state NMR spectroscopy is the unique method of choice to study these insoluble, noncrystalline, membrane-peptide complexes. Here I discuss solid-state NMR studies of antimicrobial peptides that have reported high-resolution structure, dynamics, orientation, and oligomeric states of antimicrobial peptides in a membrane environment, and also address important questions about the mechanism of action at atomic-level resolution. Increasing number of solid-state NMR applications to antimicrobial peptides are expected in the near future, as these compounds are promising candidates to overcome ever-increasing antibiotic resistance problem and are well-suited for the development and applications of solid-state NMR techniques.

1. 'Narrow lines' are beautiful but 'broad lines' are more informative

The first major step in most successful applications of NMR spectroscopy has been obtaining high-resolution spectra *even* from solid-state samples under static aligned or magic angle spinning (MAS) conditions.[1–3] The traditional appreciation for 'narrow spectral lines are most beautiful' is because high-resolution spectra considerably simplify the task of interpreting data from most systems that have been investigated thus far. As a result, high-resolution structural studies of NMR-friendly systems like water-soluble (or globular) proteins using well-established solution NMR techniques have been highly successful and recent studies have shown that it is relatively easy to extend such approaches to crystalline biomolecules that would provide 'solution-like narrow spectral lines' under MAS conditions. However, it was recently realized that a few selected NMR techniques could reveal dynamical structures of molecules that are traditionally considered to be 'messy' and challenging to most physical techniques. [3–8] Such atomic-level information are highly valuable in understanding the function of a variety of biomacromolecules, whose structures are highly essential in making important advances in structural genomics, such as those found in the cell membrane interface. Unfortunately, such NMR studies are challenging due to various factors: these 'messy' molecules are non-soluble, non-crystalline, sometimes amorphous, unfriendly to most NMR techniques (for example, unstable, unavailable in abundant quantity, power-lossy, not sufficiently sensitive to NMR measurements), and spectra are typically consist of broad lines. The broad spectral lines nevertheless possess googolplex of secrets regarding the functional molecules and can no longer be ignored, however may be the challenge, if NMR spectroscopy

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needs to be a valuable tool in addressing important biological questions. Therefore, it is urgent to critically analyze the existing NMR approaches to further develop novel NMR techniques to advance structural studies on this exciting class of biomolecules that are located in a non-NMR friendly 'messy' but biologically important environment. In this article, recent high-resolution NMR studies on messy membrane-associated antimicrobial peptides and related challenges are presented.

2. What are Antimicrobial peptides and why are they important?

The emergence of increasing numbers of bacterial strains resistant to existing conventional antibiotics, that have provided safety for more than half a century, necessitates the development of new active therapeutic agents.[9] Recent surveys report that the common antibacterials such as penicillin and tetracycline are already less effective, and that bacterial strains residing in hospitals are developing resistance against vancomycin, which is now one of our last lines of defense. Naturally occurring antimicrobial peptides probably represent one of the very first evolved forms of chemical defense of living eukaryotic cells against invasion by bacteria, protozoa, fungi, and virus. Surprisingly, they must be called upon again as a last line of defense in the present. Antimicrobial peptides have survived in this role over evolutionary time scales, likely due to the fact that they are less susceptible to the development of bacterial resistance because they disrupt the membrane of bacteria through non-specific peptide-lipid interactions. [10–13] Bacteria are incapable of counteracting this easily by mutating membrane structure. The urgent need for new antibiotics has stimulated interest in the development of antimicrobial peptides as human therapeutics and amplified interest in the development of biophysical approaches to probe their mechanism. These short cationic peptides target the cell membrane of invading microorganisms leading to cell lysis and death. Development of topically applied agents, such as pexiganan63 (MSI-78) (designed by the Genaera pharmaceuticals and is an analog of the naturally occurring magainin2, extracted from the skin of the African frog *Xenopus Laevis*) has been the focus of pharmaceutical development largely because of the relative safety of topical therapy and the uncertainty surrounding the long-term toxicology of any new class of drug administered systemically.[14,15] The main hurdle that has hindered the development of antimicrobial peptides is that many of the naturally occurring peptides (such as magainin), although active *in vitro*, are effective in animal models of infection only at very high doses, often close to the toxic doses of the peptide, reflecting an unacceptable margin of safety. MSI-78 did not pass the FDA approval (and is pending for further studies), but still is one of the promising AMPs to date for therapeutic purposes.[14]

3. Need for atomic-level resolution studies on Antimicrobial peptides at the membrane interface

While AMPs have survived the bacterial resistance problem, the development of more potent and highly selective AMPs for pharmaceutical applications necessitates the atomic-level understanding on their mechanism of action.[11] Although the amino acid sequences of different antimicrobial peptides are highly heterogeneous, they are generally cationic and amphipathic. Their amphipathicity is enhanced upon the induction of specific secondary structures, such as α -helices, β -sheets, or extended polyproline-like helices and this amphipathicity is thought to play a key role in their antimicrobial mechanism of action.[10, 13] Their general mechanism of action is permeabilization of the cell membrane, but how this is accomplished, as well as how structural and dynamical variations affect the efficacy of membrane permeation is unclear (see Figure 1). Partly due to the diversity of antimicrobial peptides now known, there is no general agreement for the molecular mechanisms underlying their antimicrobial activity apart from assuming the induction of perturbations in the membranes of target cells. These structural perturbations vary from peptide-lined pore formation to a carpeting mechanism causing toroidal punctures and additional mechanisms

may also exist. Moreover, a single peptide may employ different mechanisms of perturbation depending on the membrane lipid composition.[12,13] Therefore, in order to obtain a detailed understanding of how antimicrobial peptides carry out their broad-spectrum bactericidal activity and how these functions can be altered for biomedical or biotechnological purposes, it is necessary to determine the molecular events at very high-resolution in the mechanistic pathway of an AMP. For example, atomic-level resolution information on the secondary and tertiary structure, dynamics, and oligomeric state of an AMP in solution and also in a membrane-like environment, and the peptide orientation in membrane, is essential to completely understand the potency and selectivity of an AMP. Such information will provide insights into the role of individual amino acids, net charge of the peptide, distribution of charged residues in the peptide, peptide length, secondary structure, and hydrophobic/amphipathic angle of the secondary structure of the peptide.

4. Antimicrobial peptides – tiny but smart bombs that pose tremendous challenges to NMR

Ever since the introduction of 2D NMR pulse sequences, high-resolution structures of biomolecules have been flooding the literature and protein data bank. It has now become so routine that solving the 3D structure of a well-behaved globular protein is as easy as obtaining the structure from a high quality single crystal of a protein using X-ray crystallography. However, an NMR study of a membrane-associated peptide in lipid bilayers can be more challenging than that of a large (~40 kDa) globular protein.[11] Recent studies have shown that the above-mentioned challenges are very common when dealing with membrane-associated peptides such as antimicrobial peptides, toxins, fusion peptides, and amyloid peptides. Since these molecules function by permeabilizing the structure of the cell membrane, they pose additional challenges for NMR measurements. For example, physiologically relevant experimental conditions necessary to provide meaningful insights into the biological function of these exciting classes of molecules may not be suitable for NMR measurements. As a result the commonly used approaches of modifying the sample condition/preparation to obtain narrow spectral lines can provide misleading results and may not address biological questions no matter how beautiful the high-resolution spectra are. Because of these challenges, membrane-associated peptides are increasingly being used to develop new NMR methods that can be useful to study large-size membrane proteins. Recent studies have demonstrated that a combination of solid-state NMR techniques can be used to determine high-resolution dynamical structural images of these molecules providing insights into their biological function (Figure 2). Successful advances in NMR studies of antimicrobial peptides are presented below (see Figure 2).

5. Significant biological questions addressed by solid-state NMR studies on antimicrobial peptides

Structural studies

Atomic-level high-resolution structural information about an AMP in solution and membrane-like environment is essential in evaluating its potency and selectivity, and in designing more potent AMPs for pharmaceutical applications. However, obtaining such information accurately is a major challenge to most biophysical techniques. It cannot be obtained from the most commonly used biophysical techniques such as X-ray diffraction as the conditions under which the measurements need to be made are not favorable for this technique. Other biophysical techniques like circular dichroism, fluorescence, and IR can only provide low-resolution information but the biological relevancy of fluorescence measurements is questionable due to the changes induced by the fluorescent label. Solution NMR experiments have been used to solve 3D structures of AMPs in solution and in well-behaved detergent micelles. Most linear

AMPs are unstructured in solution; an exception is LL-37, a human cathelicidin AMP, which forms a helical structure at a high peptide concentration or in the presence of salt. Recent studies have reported highly helical structure and depth of insertion of amphipathic LL-37 [16–19], pardaxin [20,21], MSI-78 [22–24], and MSI-594 [23,24] antimicrobial peptides in detergent micelles. It was found that these linear cationic peptides are located close to the surface of the micelle. Interestingly, MSI-78 was found to form an antiparallel helical dimer [24] like the naturally occurring magainin2 [25] but at a low concentration making MSI-78 an excellent candidate to treat bacterial infections. Since the formation of dimer increases the charged surface area to interact with the anionic lipid membrane bacteria, the potency and selectivity of MSI-78 is much higher than other designed peptides. High-resolution structures determined from micelles are particularly useful in understanding the role of amphipathicity of the structure, the hydrophobic angle, and side-chain geometry. In favorable cases, it is possible to investigate intermolecular interactions. However, lack of native membrane components in micelles and its curvature-imposed effects on the structure and orientation of an AMP are main limitations of such solution NMR studies in micelles. In addition, the size and oligomeric state of some AMPs limit the application of solution NMR spectroscopy to study AMPs. On the other hand, solid-state NMR spectroscopy, fortunately, is neither limited by the size nor by the state of the system under investigation. Therefore, solid-state NMR experiments can be used to solve the structure of the peptide in solution (mostly under frozen conditions) and in near-native model membranes irrespective of the oligomeric size and sample conditions. Several original articles and reviews on the details of solid-state NMR techniques, sample preparations, approaches to measure NMR parameters that address significant biological problems, and interpretation of NMR data with regard to biological questions have recently appeared in the literature. Therefore, only a very brief summary of key NMR approaches and significant findings in the field is summarized below.

Solid-state NMR techniques to measure dipolar couplings under MAS have been used to determine the backbone conformation of AMPs in MLVs. Particularly, REDOR [26] is the most robust technique for such studies and also to investigate intermolecular interactions. [27] On the other hand, solid-state NMR experiments on aligned samples have been well utilized to determine the topology and dynamics of an AMP at atomic-resolution in bilayers with various combinations of membrane components.[16,20,22,23,28,29] Difficulties in preparing stable and reliable mechanically aligned lipid bilayers have been overcome using a sublimable solid like naphthalene.[30] This approach enabled the alignment of various combinations of membrane components to mimic bacterial vs mammalian membranes.[11] Solid-state NMR experiments have shown changes in the membrane orientation of an antimicrobial peptide when the composition of the membrane is varied. For example, pardaxin has a transmembrane orientation in thinner DMPC bilayers while it is oriented near the bilayer surface in thicker POPC bilayers.[20,21] This effect is attributed to the hydrophobic mismatch between the hydrophobic length of the peptide and the hydrophobic thickness of the POPC bilayer as observed for other transmembrane peptides [31–33]. These results are in excellent agreement with its ion-channel formation in certain lipid composition and fusogenic activity in other type of membranes. Studies on PGLa and magainin2 reported changes in the membrane orientation due to peptide-peptide interactions [34]. High-resolution dynamical images obtained from 2D PISEMA [4,35,36] and HIMSELF [37] experiments on aligned samples have provided valuable insights into the mechanism of several AMPs. For example, experimental data from aligned samples were used to rule out the barrel-stave type mechanism of membrane disruption by MSI-78 [23], MSI-594 [23], MSI-843 [38], PGLa [39], LL-37 [11,16,17], subtilosin A [40], granulysin [41], and several other AMPs. These structural studies utilized AMPs selectively labeled with a suitable combination of ^{13}C , ^{15}N , ^2H , and ^{19}F isotopes.

Peptide-induced disorder in membrane

Since most AMPs function by permeabilizing bacterial cell membranes, it is important to understand the peptide-induced structural and/or phase changes to the surrounding molecules such as lipids, lipopolysaccharides, and cholesterol [42]. Indeed, it is not surprising that the membrane components play vital role in the potency and selectivity of an AMP. For example, determining the role of anionic lipids and cholesterol can provide insights into the mechanism by which an AMP permeabilizes (or sometimes disrupts) the inner membrane of bacteria and also on the selectivity of an AMP. The role of LPS-peptide interaction is useful in understanding the activity of an AMP against Gram-negative bacteria [43]. While differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), and fluorescence experiments can be used to obtain valuable low-resolution information, solid-state NMR has been the only biophysical technique capable of providing biologically-relevant high-resolution insights into the peptide-induced changes in the membrane.[16,21] As demonstrated in our studies, one of the major advantages of using solid-state NMR spectroscopy is the ability to use different types of fully-hydrated model membranes to mimic inner or outer membrane of bacteria or mammalian cell membranes. Since solid-state NMR experiments are not limited by the composition of the model membranes used, the role of individual membrane-components can be investigated. NMR lineshapes can be correlated with dynamics, disorder/order, structural inhomogeneity, lipid phase, and membrane-curvature.[11,22] Structural information on the membrane bilayers can be obtained from the same sample that is used to determine the peptide structure, dynamics, and membrane orientation and insertion. Thus, solid-state NMR spectroscopy is a highly valuable tool in the investigation of antimicrobial peptides and the approaches developed in this field are also applied to study other complex biological systems like toxins, amyloid peptides, fusions peptides, dendrimeric compounds, etc.

A combination of unaligned MLVs and aligned lipid bilayers has been used to measure ^{31}P , ^{14}N , and ^2H NMR parameters. These parameters provide insights into the peptide-induced structural and dynamical changes in different regions of the lipid bilayer. Studies have shown that ^{31}P chemical shift and ^{14}N quadrupole coupling spectra of well-aligned samples are easy to obtain and revealed the changes in the lipid head group conformation due to AMP interaction with membrane.[19] ^{31}P chemical shift line shapes of phospholipids bilayers can be used to differentiate lamellar phase lipid bilayer structure from AMP-induced non-lamellar phase structures like hexagonal phase, cubic phase, small fragments, micelles, and bicelle type disks. For example, ^{31}P solid-state NMR studies on aligned POPE bilayers containing AMPs such as MSI-78 [22], MSI-598 [23], pardaxin [20], or LL-37 [16] have been used to measure the dependence of the lamellar to inverted hexagonal phase transition temperature of POPE on the peptide concentration. These studies have reported the peptide-induced curvature strain on membrane bilayers, which is a key parameter in determining the mechanism of membrane permeabilization by AMPs. It has been reported that pardaxin induces negative curvature strain while all other above-mentioned peptides induce positive curvature strain on lipid bilayers. [20,44] Identification of non-lamellar phases in the presence of MSI peptides was used to explain the toroidal-pore mechanism at low peptide concentration and detergent-type bicellization mechanism at high peptide concentration. The formation of micelles or small lipid fragments due to peptide interaction with membrane as indicated by an isotropic ^{31}P chemical shift peak also reported for samples containing MSI peptides when they are equilibrated for more than a month time. ^{14}N quadrupole coupling spectra measured from choline group containing PC lipid like DMPC and POPC have been used to measure the lipid-lipid and lipid-peptide interactions near the water interface.[45] Since most AMPs are cationic, their interaction with POPC bilayers enhances the symmetry around ^{14}N in the choline group and thus reducing the observed quadrupole coupling. ^{14}N experiments are also useful to measure the peptide-induced bilayer surface charge due to oligomerization, domain formation, or structural changes.[19] Deuterated lipids have been used to determine changes in the lipid head

group conformation in the presence of AMPs and also to measure peptide-induced disorder in the hydrophobic core of lipid bilayers [17]. It has been shown that ^2H quadrupole couplings measured from the acyl chains of lipids provide insights into the toroidal-pore geometry [46]. Recently, 2D SLF experiments have been used to measure the disorder along the acyl chains of lipid bilayers at natural abundance without the need for ^2H isotope.[47] All of these measurements are easy, robust, less expensive, and have been shown to provide valuable insights into the mechanism of membrane permeabilization by AMPs.

Role of individual membrane components

The membrane-composition can alter the topology of a given peptide in the membrane, the mechanism of membrane-disruption, and the level of antimicrobial activity.[20,42] This is important in peptide selectivity because prokaryotic and eukaryotic cells differ in the composition and topological arrangement of lipids. The surface of mammalian cell membranes is composed of electrically neutral, zwitterionic phospholipids, whereas bacterial membranes contain large amounts of negatively charged phospholipids. Cationic antimicrobial peptides selectively bind and permeabilize anionic bacterial membranes. The driving forces of binding are electrostatic interactions between the positive charges of the peptides and the negative charges of the lipids, and the hydrophobic interactions between the non-polar amino acids and the hydrophobic core of the membrane. The hydrophobicity of antimicrobial peptides is generally too low to effectively associate with zwitterionic phospholipids, avoiding toxicity against the host cell. Therefore, the composition of the membrane is crucial in determining the selectivity and activity of antimicrobial peptide. Solid-state NMR studies have shown that the structure, dynamics, and membrane orientation of an AMP depends on the composition of membranes. For example, it has been shown that the function of pardaxin is membrane-composition dependent using solid-state NMR studies on aligned model membranes.[20] It has also been shown that cholesterol inhibits the function of this peptide by preventing the formation of peptide-induced, non-lamellar phase lipid domains; also the variation in the lipid charge and headgroup size alters the peptide function. The dependency of the toroidal-pore formation by MSI-78 on the membrane-composition has also been reported.[22]

Oligomeric structures of antimicrobial peptides

Peptide-peptide interaction either in solution or in membrane is the key step in oligomerization of antimicrobial peptides. While most linear AMPs are unstructured and exist as monomers in solution (an exception is LL-37), oligomers present in membrane can be heterogeneous and the size of oligomers can vary depending on a variety of parameters such as: peptide concentration, membrane composition, ionic concentration, pH, and temperature.[19,48] It may be possible to prepare samples that contain homogeneous oligomers that can result in high-resolution NMR spectra lines; while studies on such samples are definitely of academic importance they could be less significant as it may not reflect the physiological situation where a dynamical exchange among heterogeneous oligomeric states plays important roles in the biological function of AMPs. Therefore, it is important to characterize the oligomeric structures under biologically-relevant conditions. Solid-state NMR techniques are capable of revealing the oligomeric structures, but caution must be exercised in preparing the samples. Characterization of oligomers is extremely difficult, and therefore only a handful of studies have been successful. NMR studies have shown that MSI-78 and magainin2 exist in antiparallel dimeric helical structures.[24,25] Another study has shown that LL-37 exists in heterogeneous oligomeric conditions both in solution and membrane. Synergistic effects based on the heterodimers of magainin2 and PGLa have been reported. Oligomeric forms of protegrin have also been reported from solid-state NMR measurements.[49] These studies have shown that ^{14}N , ^2H and ^{19}F NMR experiments are useful in the characterization of oligomers. However, the intrinsic difficulties in determining oligomeric structures demand better solid-state NMR techniques.

6. Future challenges

An increasing number of solid-state NMR studies on AMPs reported in the literature indicate the significance of the biological problem and the power of solid-state NMR techniques to investigate the atomic-level resolution of these exciting molecules in messy environments. While solid-state NMR studies have already addressed a number of significant biological questions related to antimicrobial peptides, there is a need for more sophisticated techniques for various reasons mentioned below. (1) Development of robust and high throughput solid-state NMR methods would enable studies on a variety of AMPs. Such methods will find applications in studying other class of membrane-associated molecules like toxins, amyloid peptides/proteins, fusion peptides/proteins, and polymeric compounds like dendrimers and other designed compounds. (2) Existing solid-state NMR experiments demand a large quantity of sample for investigation.[50] Therefore, there is need for techniques with very high sensitivity for measurements at the minimum inhibitory concentrations of AMPs. (3) It would be useful to develop approaches for *in cell* NMR measurements. Such experiments can provide insights into structures and properties of AMPs in native conditions. (4) Since freezing lipid bilayers containing AMPs to suppress dynamics and to enhance sensitivity may not be suitable to address important biological questions, there is need for NMR techniques that can be used for measurement at 37°C. Since a cell membrane at this temperature is a liquid crystalline solid, such techniques could be developed based on a simple combination of existing solution and solid-state NMR principles. In this context, recently developed COMPOZER-based techniques are promising, and use of residual dipolar couplings, residual chemical shift anisotropy, and residual quadrupole coupling parameters would provide valuable insights into the biological pathways of AMPs. (5) Development of approaches to measure atomic-level dynamics at various time scales will be valuable in examining the mechanistic dynamical pathways that lead to folding, refolding based on membrane composition, misfolding, oligomerization, and in some cases fibril formation. The realization of many functional roles and promises of AMPs in pharmaceutical applications, and the power of solid-state NMR methods to investigate AMPs in near-native environments will ought to lead to a myriad of research activities in this exciting area. (6) High throughput solid-state NMR methods are essential to quickly measure the action of peptides in membranes as increasing number of exciting studies on the design of antimicrobial peptides.[14,15,51–53]

The advent of higher magnetic fields, advancements in sophisticated instrumentation, the development of more near-native model membranes, the availability of a variety of well-studied membrane-associated peptides and proteins, the increasing number of membrane studies utilizing solid-state NMR along with other low-resolution biophysical techniques to provide a better picture of a chosen biological problem [54–56], and the merging solid and solution NMR fields are all exciting activities with encouraging trends and will continue to increase the power of solid-state NMR to study even more challenging membrane-related biological problems.

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Biography

Professor Ayyalusamy Ramamoorthy obtained his PhD in Chemistry in 1990 from the Indian Institute of Technology (Kanpur, India) working on the development of composite pulse sequences for studies using NQR spectroscopy. He subsequently moved to the Central Leather Research Institute (Madras, India) as a Fellow Scientist to develop scalar coupling based NMR methods for structural studies using solution NMR spectroscopy. In 1992, he joined JEOL Ltd (Tokyo, Japan) as a Research Scientist in the laboratory of Professor Kuniaki Nagayama to develop recoupling techniques (including USEME and *J*-HOHAHA) for magic angle spinning NMR studies on biosolids. He then joined the Stanley Opella group (Department of Chemistry, University of Pennsylvania, Philadelphia) in 1993 to further develop and apply solid-state

NMR techniques (including PISEMA, PSEUDO and PISEMAMAT) for structural studies on membrane proteins. In 1996, he joined the University of Michigan in Ann Arbor where he currently holds a joint appointment as Professor in Biophysics and Department of Chemistry. His main research interests are on the development and applications of solid-state NMR spectroscopy to study the structure, dynamics and function of membrane protein complexes, antimicrobial peptides, and amyloid peptides.

Figure 1. Biological pathways representing biochemical and functional properties of cationic antimicrobial peptides

Understanding the events in the mechanism of an AMP is a complex problem and a major challenge to existing biophysical and biochemical tools. There are a number of features worth mentioning. Step 1 could be the key in escaping from enzymatic degradation and it depends on the concentration of peptide and ions, and pH. Fibril formation of an AMP, reported for LL-37, could be important in the stabilization of AMPs so that they can be available when required to fight against infection. Steps 2 and 3 could be considerably different. Step 2 involves a combination of the formation of a minimum energy amphipathic structure, enthalpy-driven membrane binding and folding processes. On the other hand, step 3 is a combination of refolding of the oligomeric structure, enthalpy-driven binding and folding processes. In addition, steps 2, 3 and 4 highly depend on the membrane composition. AMPs could cause cell death by any one of the mechanisms and/or by just gently permeabilizing to alter the voltage across the membrane. There are many exciting questions still need to be addressed. What roles fibrils play in the function of an AMP? Are fibrils of an AMP toxic? Are there any similarities between AMP fibrils and amyloid fibrils? Are the size and structure of AMP oligomers in solution and membrane different? To what extent the oligomeric size, structure, orientation, and depth of insertion in membrane depend on the membrane composition, peptide concentration and temperature? While the structure and organization of lipids vary, how different are the various types of membrane disrupting mechanisms energetically? Are some of these structures kinetically trapped? What causes one type of mechanism to change to another for a given peptide? It is well established that the conformation, dynamics, and phase of lipids depend on the temperature. Does freezing the model membrane containing an AMP for characterization using solid-state NMR experiments alter the peptide structure, peptide-peptide interactions, peptide-lipid interaction, peptide-water-lipid interactions, membrane

orientation, oligomeric size, pore geometry, etc. How does an AMP pass through the outer membrane of a Gram-negative bacteria? What type of interactions and structures expected when an AMP enters the cell wall? How similar/different are the structural measurements obtained from model membranes and living cells? These questions are difficult to be addressed satisfactorily as the amino acid composition of an AMP also plays a role and complicates the efforts in seeking for a general answer. Nevertheless, solid-state NMR spectroscopy is the only technique that can provide accurate answers to most of these questions, and is highly valuable in providing insights at each and every stage of this biochemical pathway as mentioned in the text. In addition, solid-state NMR measurements combined with results from calorimetric experiments, fluorescence experiments, and molecular dynamics simulations could be more valuable to satisfactorily address this complex biological problem. It should be noted that some AMPs have been shown to function by interacting with receptor proteins in membranes or by permeating the cell membrane and interfering with RNA function, which are not covered in this review.

Figure 2. A summary of advantages and concerns of various NMR methods and model membranes used to study antimicrobial peptides

A combination of different types of NMR approaches and suitably selected model membranes can address biological questions with regard to the function of AMPs as discussed in the text.