

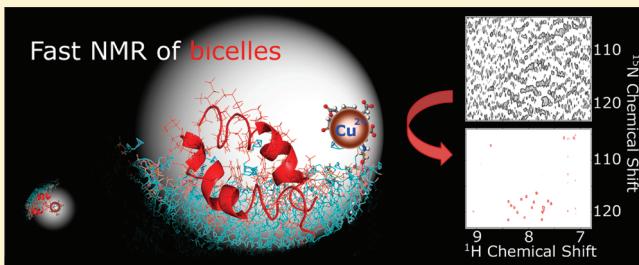
# Fast NMR Data Acquisition From Bicelles Containing a Membrane-Associated Peptide at Natural-Abundance

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 Supporting Information

**ABSTRACT:** In spite of recent technological advances in NMR spectroscopy, its low sensitivity continues to be a major limitation particularly for the structural studies of membrane proteins. The need for a large quantity of a membrane protein and acquisition of NMR data for a long duration are not desirable. Therefore, there is considerable interest in the development of methods to speed up the NMR data acquisition from model membrane samples. In this study, we demonstrate the feasibility of acquiring two-dimensional spectra of an antimicrobial peptide (MSI-78; also known as pexiganan) embedded in isotropic bicelles using natural-abundance  $^{15}\text{N}$  nuclei. A copper-chelated lipid embedded in bicelles is used to speed-up the spin–lattice relaxation of protons without affecting the spectral resolution and thus enabling fast data acquisition. Our results suggest that even a 2D SOFAST-HMQC spectrum can be obtained four times faster using a very small amount ( $\sim 3\text{ mM}$ ) of a copper-chelated lipid. These results demonstrate that this approach will be useful in the structural studies of membrane-associated peptides and proteins without the need for isotopic enrichment for solution NMR studies.



High-resolution structural and dynamical studies of membrane proteins and peptides are becoming increasingly important as the ability to visualize atomic-level details provides powerful insights into their functional properties. Despite the challenges posed by membrane proteins, NMR spectroscopy has been successfully utilized recently to provide a wealth of atomic-level resolution structural and dynamical information from a variety of model membranes.<sup>1–9</sup> However, in spite of the recent technical advances, relatively poor sensitivity of NMR spectroscopy continues to be a major bottleneck for high-throughput applications.<sup>10–13</sup> Specifically, stringent requirements on the quantity and stability of a sample and the long data acquisition process are not suitable for most membrane proteins that are scarcely available and/or their production could be very expensive. It is also not desirable to enhance the S/N by increasing the concentration of membrane active molecules such as antimicrobial peptides, amyloid peptides, toxins, and fusogenic peptides as they may oligomerize to disrupt the membrane.<sup>14–16</sup> The mandatory requirement for isotopic labeling of membrane proteins further limits NMR applications, as there are numerous molecules that cannot easily be obtained biologically. Therefore, there is considerable interest in the development of novel approaches to study unlabeled proteins and also to speed up the NMR data acquisition process. In this study, we demonstrate an approach for NMR structural studies of a membrane protein embedded in bicelles without the need for isotopic enrichment by speeding up the spin–lattice relaxation process for protons.

## INTRODUCTION

High-resolution structural and dynamical studies of membrane proteins and peptides are becoming increasingly important as the ability to visualize atomic-level details provides powerful insights into their functional properties. Despite the challenges posed by membrane proteins, NMR spectroscopy has been successfully utilized recently to provide a wealth of atomic-level resolution structural and dynamical information from a variety of model membranes.<sup>1–9</sup> However, in spite of the recent technical advances, relatively poor sensitivity of NMR spectroscopy continues to be a major bottleneck for high-throughput applications.<sup>10–13</sup> Specifically, stringent requirements on the quantity and stability of a sample and the long data acquisition process are not suitable for most membrane proteins that are scarcely available and/or their production could be very expensive. It is also not desirable to enhance the S/N by increasing the concentration of membrane active molecules such as antimicrobial peptides, amyloid peptides, toxins, and fusogenic peptides as they may oligomerize to disrupt the membrane.<sup>14–16</sup> The mandatory requirement for isotopic labeling of membrane proteins further limits NMR applications, as there are numerous molecules that cannot easily be obtained biologically. Therefore, there is considerable interest in the development of novel approaches to study unlabeled proteins and also to speed up the NMR data acquisition process. In this study, we demonstrate an approach for NMR structural studies of a membrane protein embedded in bicelles without the need for isotopic enrichment by speeding up the spin–lattice relaxation process for protons.

Our experimental results show that it is possible to obtain high-resolution multidimensional spectra from a membrane-associated peptide (MSI-78,<sup>17</sup> Figure 1) at natural-abundance of  $^{15}\text{N}$  nuclei and the data can be collected four times faster, even when the SOFAST-HMQC<sup>18</sup> experiment is employed, using isotropic bicelles containing a copper-chelated lipid (Figure 2). Since isotropic bicelles tumble fast enough to result in narrow spectral lines and used in the structural studies of peptides and proteins, DMPC/DHPC bicelles with  $q$  ratios 0.5 and 0.25 ( $q = [\text{DMPC}]/[\text{DHPC}]$ ) containing MSI-78 were used in this study for solution NMR measurements. MSI-78 is a 22-residues (a molecular weight of 2477.20 Da), linear, cationic antimicrobial peptide (Figure 1). Details on the synthesis and purification of MSI-78 can be found elsewhere.<sup>19</sup> 3D structure of dimeric form of MSI-78 embedded in DPC micelles (Figure 5B) determined from NMR experiments has been reported.<sup>20</sup> As shown in Figure 1, MSI-78 was designed to exhibit an amphipathic  $\alpha$ -helical structure between residues 4 and 19 (highlighted in red) in a membrane environment, whereas it is unstructured in solution.<sup>19</sup> Solid-state NMR studies reported the lipid-peptide interactions, membrane-associated structure, and its mechanism of membrane disruption of MSI-78.<sup>21,22</sup>

Previous solution and solid-state NMR studies have used paramagnetic salts (such as copper-EDTA) to speed up the spin–lattice ( $T_1$ ) relaxation rate of protons in solution as well as

**Received:** August 8, 2011

**Revised:** September 19, 2011

**Published:** September 22, 2011



Figure 1. Amino acid sequence of a linear, cationic antimicrobial peptide MSI-78 (also known as pexiganan).

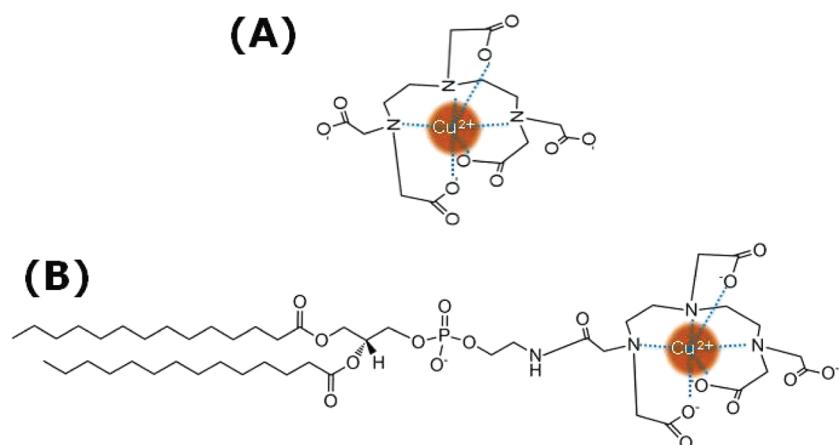


Figure 2. (A) Molecular structure of DTPA (diethylenetriaminepentaacetic acid) chelated with a copper ion. DTPA is one of the common metal ion chelators. (B) The structure of DMPE-DTPA (1,2-ditetradecanoyl-sn-glycero-3-phosphoethanolamine-N-diethylenetriaminepentaacetic acid). The free diffusion of copper-DTPA metal complex is restricted and immobilized as the DMPE lipid is anchored in lipid bilayers.

in solid-state samples.<sup>13,23–32</sup> Solution NMR studies focused on water-soluble proteins while solid-state NMR studies utilized paramagnetic salts on crystalline biomolecules.  $T_1$  of  $^{13}C$  nuclei was shortened in carbon-detected solution NMR experiments using  $Gd^{3+}$ .<sup>33</sup> Later, Wuthrich and co-workers demonstrated the significant increase in the signal-to-noise ratio of the labile amide protons by reducing the  $T_1$  of water protons from 3 to 0.3 s using 1 mM  $Gd^{3+}$ .<sup>34</sup> Since the line broadening effects due to  $Gd^{3+}$ 's long (nano- to microseconds) electronic relaxation times ( $T_{1e}$  and  $T_{2e}$ ) are not suitable for proton-detected experiments,  $Ni^{2+}$  that has a shorter relaxation time (pico to nano seconds)<sup>35</sup> was used to shorten the  $T_1$  of protons from macromolecules and water without a line broadening effect.<sup>30</sup> Recently, Ishii and co-workers have demonstrated the use of  $Cu^{2+}$  to shorten the  $T_1$  of protons in magic angle spinning (MAS) experiments on crystalline samples and enhancement in S/N by rapid data collection.<sup>25,26</sup> Reif and co-workers have demonstrated further signal enhancement because of  $T_1$  shortening by using  $Cu^{2+}$  paramagnetic effect in deuterated proteins in which labile protons are back-exchanged from  $H_2O/D_2O$ .<sup>29</sup> A recent solid-state MAS NMR study by Jaroniec and co-workers have shown rapid data collection by covalently bound paramagnetic tags in crystalline proteins.<sup>31</sup> Other solution<sup>36–42</sup> and solid-state NMR<sup>43,44</sup> studies utilized paramagnetic relaxation enhancement in metalloproteins or proteins containing a paramagnetic label. Theoretical details on the paramagnetic effect on  $T_1$  and  $T_2$  and also on the shift in resonances for various ions can be found in these previous studies. Recent ultrafast MAS studies demonstrated the use of a paramagnetic metal center in a protein to assign resonances in uniformly labeled crystalline proteins.

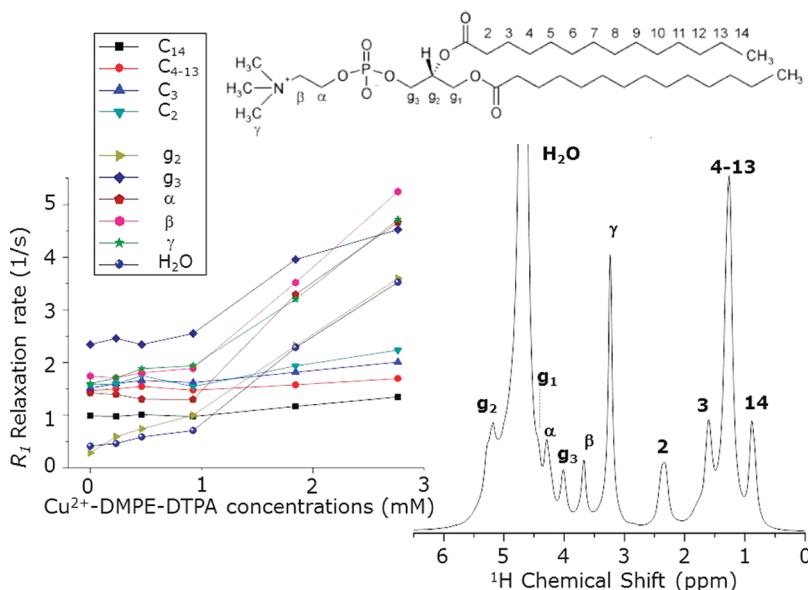
While previous studies have thoroughly investigated on the use of a paramagnetic salt to shorten the  $T_1$  of a water-soluble protein in solution NMR structural studies and a crystalline protein in MAS solid-state NMR studies, extending this approach

to study a membrane protein is difficult. We have recently demonstrated the use of a copper-chelated lipid to reduce the  $T_1$  of protons from lipid bilayer samples. Since the freely diffusing copper-EDTA molecules in solution are inefficient in augmenting the  $T_1$  process, it is essential to use a large amount (>30 mM) of paramagnetic ions in the sample.<sup>25</sup> In this study, a significantly smaller amount of copper-chelated phospholipid (DMPE-DTPA: 1,2-ditetradecanoyl-sn-glycero-3-phosphoethanolamine-N-diethylenetriaminepentaacetic acid (Figure 2)) was homogeneously mixed with DMPC:DHPC bicelles. Three main advantages of using a copper-chelated lipid are (i) approximately a 10-times lower paramagnetic ion concentration was sufficient to accelerate the spin-lattice relaxation rate ( $R_1$ ) than previous reports;<sup>24–32</sup> (ii) it can be mixed homogeneously without altering the properties of other components of bicelles; and (iii) the significant reduction in the required amount of the copper-chelated lipid considerably reduces the sample heating effect due to radio frequency field and enables faster NMR data collection.

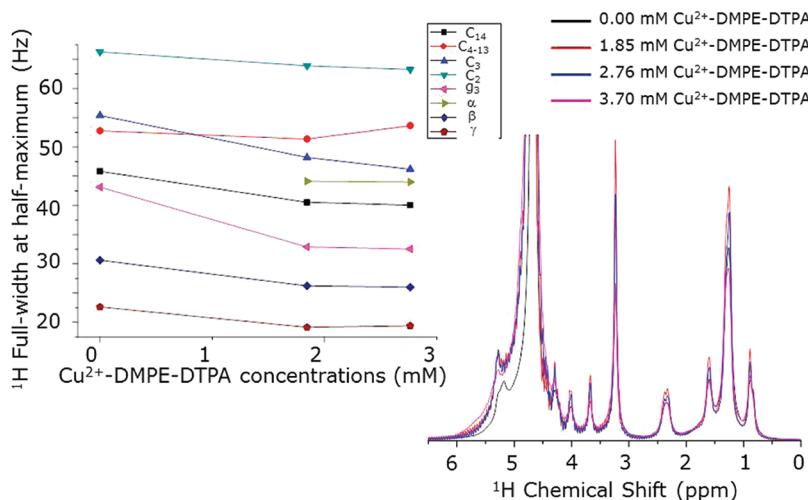
## EXPERIMENTAL SECTION

**Preparation of Isotropic Bicelles Containing a Copper-Chelated Lipid for NMR Experiments.** Isotropic bicelles containing a longer acyl chain phospholipid (DMPC = 1,2-dimyristoyl-sn-glycero-3-phosphocholine used in the present study) and a detergent (DHPC = 1,2-diheptanoyl-sn-glycero-3-phosphocholine used in the present study) with and without DMPE-DTPA chelator lipid were used in this study. DMPC, DHPC, and DMPE-DTPA were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

DMPC/DHPC bicelles<sup>45–51</sup> were prepared using the following procedure. In the case of  $q = 0.5$  bicelles, 55.0 mg of DMPC



**Figure 3.** Spin–lattice relaxation rate ( $R_1$ ) of protons from DMPC/DHPC isotropic bimolecules ( $q = 0.5$ ) for different concentrations of a copper-chelated lipid,  $\text{Cu}^{2+}$ -DMPE-DTPA at  $35^\circ\text{C}$ . The  $R_1$  values were determined from  $^1\text{H}$ -spin-inversion recovery experiments and the estimated error from the best-fitting of experimental data is about  $\pm 0.05$ . One-dimensional  $^1\text{H}$  chemical shift spectrum of DMPC/DHPC isotropic bimolecules with 0 mM  $\text{Cu}^{2+}$ -DMPE-DTPA at  $35^\circ\text{C}$  (bottom right). The chemical structure of DMPC is shown at the top. All measurements were performed on a 400 MHz Varian solid-state NMR spectrometer.

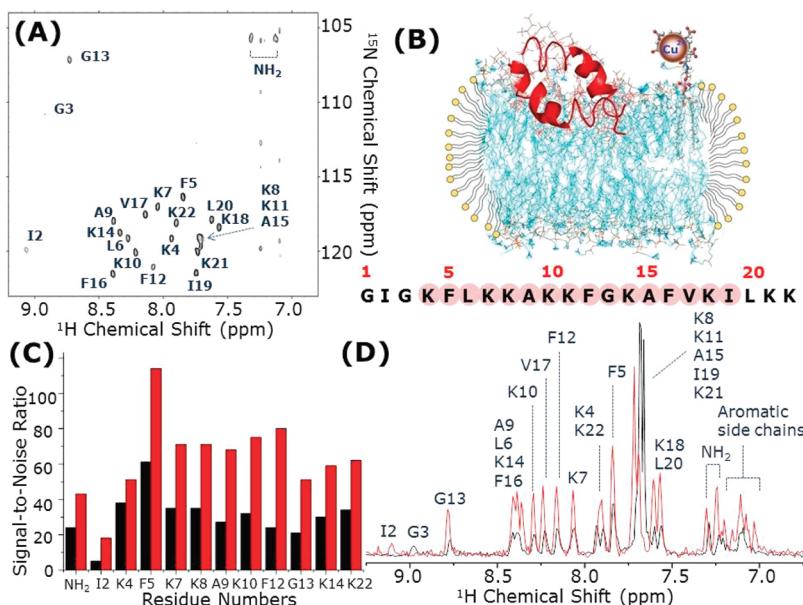


**Figure 4.** Full-width at half-maximum (FWHM) and  $^1\text{H}$  NMR spectra of DMPC/DHPC isotropic bimolecules ( $q = 0.5$ ) for different concentrations of a copper-chelated lipid,  $\text{Cu}^{2+}$ -DMPE-DTPA, at  $35^\circ\text{C}$ . The close resemblance of proton chemical shift spectra for different  $\text{Cu}^{2+}$ -DMPE-DTPA concentrations suggests that there is no change in the bimolecular properties of the samples due to the presence of  $\text{Cu}^{2+}$  ions. All measurements were carried out on a 400 MHz Varian solid-state NMR spectrometer.

and 73.6 mg of DHPC were cosolubilized in 1 mL of chloroform. Subsequently, chloroform was removed under a stream of  $\text{N}_2$  gas to form a film on the walls of a glass tube and the film was kept under vacuum overnight to remove the residual solvents. Then the lipid films were solubilized into 366  $\mu\text{L}$  of a 10 mM phosphate buffer at pH 7.4. These transparent hydrated lipid mixtures were then vortexed and homogenized by gentle sonication in an ice bath for 30 min, and subjected to more than 4 freeze/heat cycles between liquid nitrogen and  $40^\circ\text{C}$  of water. Once a satisfactory mixing of the components was completed, the sample ( $\sim 330 \mu\text{L}$ ) was transferred to a 5 mm Shigemi NMR glass tube. The isotropic bimolecule formation was examined using

$^{31}\text{P}$  NMR experiments after 30 min of incubation at  $37^\circ\text{C}$  in the NMR magnet.

Isotropic bimolecules containing MSI-78 were prepared by mixing 130.2 mg of lipids (in the case of  $q$  ratio = 0.5, 1.65 mg of DMPE-DTPA, 55.0 mg of DMPC, and 73.6 mg of DHPC) with 11.60 mg of (unlabeled) MSI-78 and following the steps as detailed above. To prepare bimolecules containing the copper-chelated lipid, 0.349 mg of  $\text{Cu}(\text{NO}_3)_2 \cdot 2\text{SH}_2\text{O}$  was added (for  $q$  ratio = 0.5 case) to a mixture of isotropic bimolecules, peptide, and the chelator lipid after adding a 10 mM phosphate buffer at pH 7.4. The final NMR sample consisted of 52:1 lipid:peptide molar ratio; the molar ratio of DMPC:



**Figure 5.** Four-fold increase in the sensitivity of 2D SOFAST-<sup>1</sup>H/<sup>15</sup>N-HMQC experiments. (A) 2D SOFAST-<sup>1</sup>H/<sup>15</sup>N-HMQC<sup>18</sup> spectrum of a 9.3 mM (unlabeled) MSI-78 (also known as pexiganan) incorporated in DMPC/DHPC isotropic bicelles ( $q = [\text{DMPC}]/[\text{DHPC}] = 0.25$ , DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DHPC: 1,2-dihexanoyl-sn-glycero-3-phosphocholine) containing a 2.96 mM copper-chelated DMPE lipid. (B) 3D structure of MSI-78 embedded in bicelles along with its amino acid sequence. Previous studies have shown that the potent antimicrobial peptide, MSI-78, is unstructured in solution and forms a helical dimer in a membrane environment.<sup>20</sup> Solid-state NMR studies have shown the membrane-surface association of this peptide and its ability to function by forming toroidal pores.<sup>22</sup> The effect of a Cu<sup>2+</sup>-chelated DMPE on the bicellar properties of DMPC:DHPC system and the paramagnetic ion induced shortening of spin-lattice relaxation time ( $T_1$ ) were examined using a series of <sup>1</sup>H NMR experiments (Figure 3 and Supporting Information Figure S1). (C) Signal-to-noise ratio obtained from 2D SOFAST-HMQC spectra of a 9.3 mM unlabeled MSI-78 in  $q = 0.5$  isotropic bicelles without copper-chelated lipid (black) and with a 2.96 mM copper-chelated lipid (red). S/N ratio and line widths were also measured for amide-NH resonances observed in 2D <sup>1</sup>H/<sup>15</sup>N SOFAST-HMQC spectra and are compared in Figures 6. Further increase in the concentration of Cu<sup>2+</sup> either did not increase the S/N or resulted in undesirable effects like line broadening (Figure 6). (D) 1D <sup>1</sup>H chemical shift projection spectrum obtained from 2D SOFAST-<sup>1</sup>H/<sup>15</sup>N-HMQC spectra that were obtained with no copper (black) and a 2.96 mM Cu<sup>2+</sup>-DMPE-DTPA (red). All spectra presented in this study were obtained from a 900 MHz Bruker NMR spectrometer at 35 °C using a cryoprobe. Each 2D SOFAST-HMQC spectrum was obtained from 64  $t_1$  experiments, 256 scans, and a 100 ms recycle delay; the total data collection time (including the acquisition time and delays in INEPT) was ~54 min. The final 2D data matrix size was 2048 × 2048 after zero-filling in both dimensions. 2D data were processed using TOPSPIN 2.1 (from Bruker). Squared sine-bell function was employed in both dimensions with a shift of  $\pi/4$ . Resonance assignment and volume fit calculations were performed using SPARKY 3.113.

Cu-DMPE-DTPA was 53.91:1 while DMPE-DTPA: Cu(NO<sub>3</sub>)<sub>2</sub> · 2.5H<sub>2</sub>O was 1: 1.

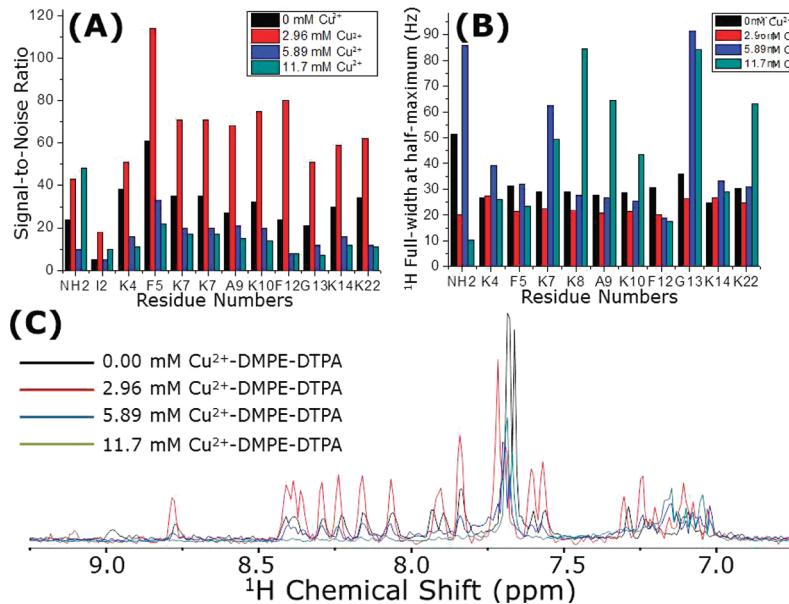
**NMR Spectroscopy.** A 400 MHz Varian solid-state NMR spectrometer and a 4 mm triple-resonance magic-angle spinning probe were used to measure spin-lattice relaxation ( $T_1$ ) relaxation of protons from bicelles for various concentrations of copper-chelated DMPE (Figures 3, and 4 and Supporting Information Figure S1) and temperature. A 4 mm glass tube containing the sample was used under the static condition. A 900 MHz Bruker NMR spectrometer equipped with a cryoprobe was used for all measurements on bicelles containing MSI-78. All other experimental details are given in the respective figure captions.

## RESULTS AND DISCUSSION

**Copper-Chelated Lipids Reduce  $T_1$  but not  $T_2$  in Isotropic Bicelles.** Since the curvature of micelles has been thought to play a role on the structural folding of certain membrane proteins, and the presence of lipids in a bicelle<sup>52–57</sup> makes it a better model membrane, we chose to use bicelles to investigate the paramagnetic effect of Cu<sup>2+</sup> for fast NMR data acquisition. Isotropic bicelles composed of DMPC, DHPC, and MSI-78 were used in NMR experiments. Since free Cu<sup>2+</sup> ions could interact with

the protein and also could result in RF-induced heating, a Cu<sup>2+</sup>-chelated DMPE lipid was used in this study. To evaluate the paramagnetic relaxation enhancement effect on isotropic bicelles due to the presence of a copper-chelated phospholipid, <sup>1</sup>H spin-inversion-recovery experiments were performed. High-resolution <sup>1</sup>H NMR spectra of isotropic bicelles enabled the measurement of site-specific  $R_1$  values as shown in Figure 3. It was found that a 2.96 mM Cu<sup>2+</sup>-chelated DMPE was sufficient to significantly shorten the  $T_1$  process down to ~0.1 s. The temperature dependence of paramagnetic relaxation enhancement effect in isotropic bicelles was evaluated by measuring  $R_1$  values by varying the temperature of the sample. As shown in Supporting Information Figure S1, the paramagnetic-induced reduction in  $T_1$  decreased when the temperature was increased, as the increasing temperature increases molecular motions in the sample. A close resemblance of <sup>1</sup>H (Figure 4) and <sup>31</sup>P (data not included) chemical shift spectra of bicelles with and without the Cu<sup>2+</sup>-chelated DMPE indicated that the insertion of copper containing DMPE lipid did not alter the DMPC/DHPC bicellar properties.

The presence of a paramagnetic ion in the sample could result in the broadening of observed spectral lines from peptide or protein embedded in bicelles. Therefore, full-width at half-maximum (FWHM) of spectral lines observed from isotropic



**Figure 6.** Signal-to-noise ratio (A) and full-width at half-maximum (FWHM) values (B) obtained from 2D SOFAST-HMQC experiments on  $q = 0.5$  isotropic bicelles containing a 9.3 mM unlabeled MSI-78 without Cu<sup>2+</sup>-DMPE-DTPA (black), and with a 2.96 mM Cu<sup>2+</sup>-DMPE-DTPA (red), 5.89 mM Cu<sup>2+</sup>-DMPE-DTPA (blue), and 11.7 mM Cu<sup>2+</sup>-DMPE-DTPA (green) at 35 °C. (C) One-dimensional <sup>1</sup>H chemical shift projections from 2D SOFAST-HMQC spectra. Other experimental and data processing details are as mentioned in the Figure 5 caption.

bicelles for different concentrations of Cu<sup>2+</sup>-DMPE-DTPA lipid were measured to evaluate the line broadening effect (Figure 4). Our results suggest that the presence of Cu<sup>2+</sup>-DMPE-DTPA up to 3 mM of concentration had no significant broadening of lipid spectral lines and therefore did not significantly alter the spin–spin relaxation ( $T_2$ ) of protons as shown in Figure 4.

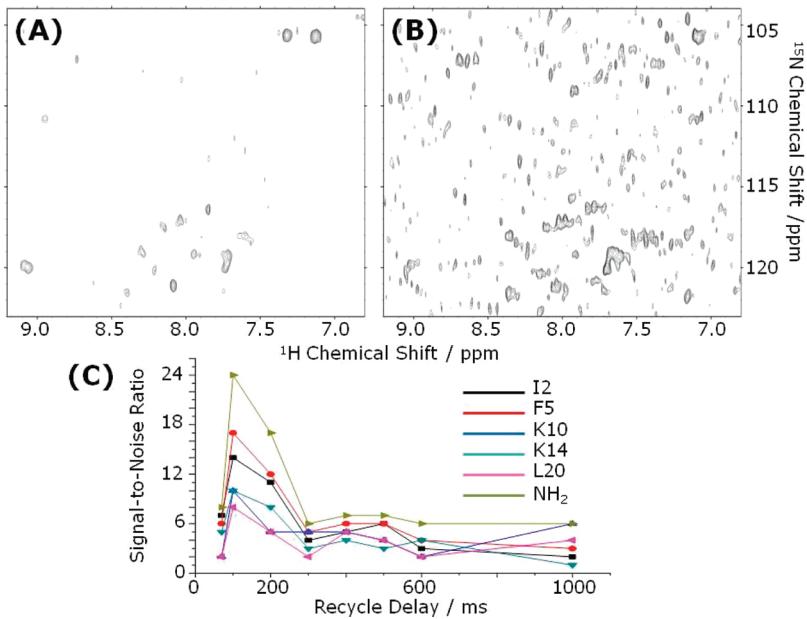
**Fast SOFAST-HMQC NMR Experiments on Isotropic Bicelles.** Sensitivity of 2D <sup>1</sup>H/<sup>15</sup>N SOFAST-HMQC experiments on bicelles containing a unlabeled MSI-78 was compared for various concentrations of Cu<sup>2+</sup>-chelated DMPE in Figure 5 (and also in Figure 6). Our results suggest that, in addition to the  $T_1$ -shortening due to the SOFAST effects, the presence of Cu<sup>2+</sup> ions further decreased the  $T_1$  values of amide-protons from MSI-78. As a result, the presence of as little as 2.96 mM Cu<sup>2+</sup> was sufficient to increase the S/N by a factor of 2 for all residues or to reduce the experimental time by a factor of ~4. Experiments were also performed on bicelles prepared with different concentrations of Cu<sup>2+</sup>-chelated DMPE. Interestingly, the use of a 2.96 mM Cu<sup>2+</sup>-chelated DMPE provided the highest sensitivity (Figures 6A and 6C) and further increase in the concentration of Cu<sup>2+</sup>-DMPE-DTPA was ineffective but resulted in line broadening as shown in Figures 6 (B) and (C). This is in agreement with a maximum  $T_1$  reduction observed for bicelles containing a 2.96 mM Cu<sup>2+</sup>-chelated DMPE (Figure 3).

Line widths of resonances observed in 2D <sup>1</sup>H/<sup>15</sup>N SOFAST-HMQC spectra of bicelles were measured. As shown in Figure 6B, no signal broadening of MSI-78 was observed in isotropic bicelles containing up to ~3 mM Cu<sup>2+</sup>-DMPE-DTPA lipid; slightly narrower peaks were observed for most sites of MSI-78 in the presence of 2.96 mM Cu<sup>2+</sup>-DMPE-DTPA lipid. On the other hand, the use of higher concentrations (5.89 and 11.7 mM) of Cu<sup>2+</sup>-DMPE-DTPA increased the line widths for several residues as shown in Figure 6B. Since the amphipathic helical MSI-78 has been reported to be associated with the lipid bilayer surface, it is possible that the  $T_2$  of hydrophilic residues K4, K7, K8, K10, and

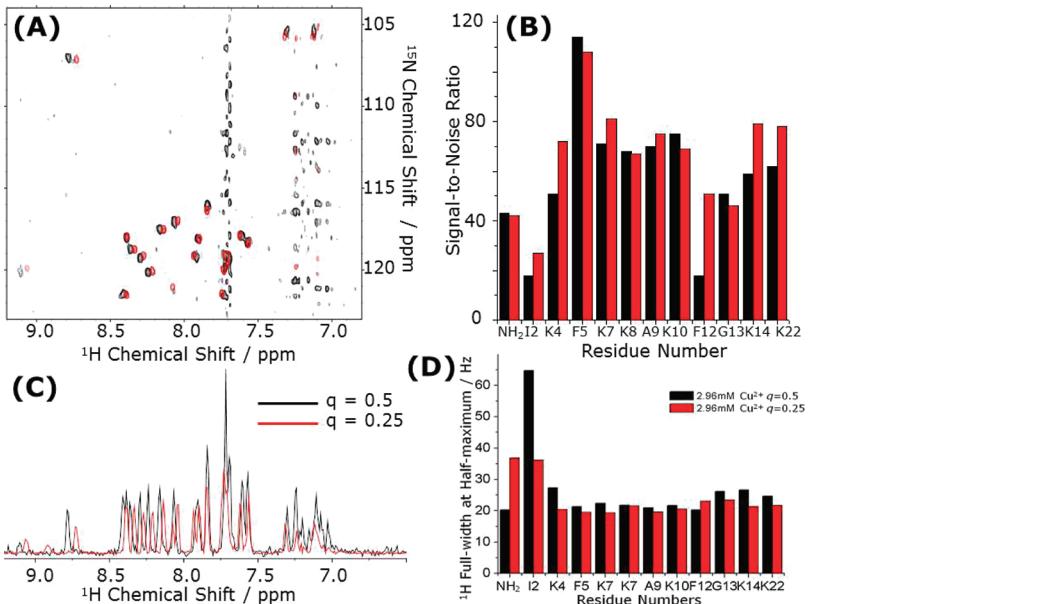
K22 exposed to the water phase are significantly affected in samples containing a high concentration of Cu<sup>2+</sup> ions. Since a concentration <3 mM Cu<sup>2+</sup>-DMPE-DTPA was sufficient to significantly reduce the  $T_1$  values and did not reduce the spectral resolution, the increase in line widths observed for higher concentrations of Cu<sup>2+</sup> ions is not a major concern for this study. However, a systematic analysis of changes in the  $T_2$  values because of the presence of Cu<sup>2+</sup>-DMPE-DTPA could provide valuable information on the topology of a membrane protein as previously demonstrated for OMPX using Gd(DOTA).<sup>42</sup> Details on the paramagnetic effects of different types of ions on  $T_1$  and  $T_2$  can be found in a recent review article.<sup>32,37</sup>

**Fast HSQC NMR Experiments on Isotropic Bicelles.** Since SOFAST-based experiments have limited applications due to the use of selective excitation RF pulses, regular 2D <sup>1</sup>H/<sup>15</sup>N HSQC experiments were performed on bicelles containing MSI-78 with no <sup>15</sup>N labeling to measure the paramagnetic ion induced sensitivity gain for various concentrations of Cu<sup>2+</sup>-chelated DMPE. Remarkably, bicelles containing the Cu<sup>2+</sup>-chelated DMPE provided HSQC spectra with a reasonable S/N within 2 h of experimental time (Figure 7A), whereas only a very noisy spectrum was obtained from a sample containing no paramagnetic ions (Figure 7B). To determine the optimized experimental conditions, a series of 2D HSQC experiments was performed by keeping the total experimental time constant and varying the recycle delay. Results compared in Figure 7C and Supporting Information Figure S2 indicate that a 100 ms recycle delay was sufficient to obtain the best S/N.

These results suggest that HSQC and possibly 3D spectra of isotropic bicelles containing an unlabeled polypeptide can be obtained; in unfavorable samples, more number of scans and increased amount of the protein can be used to obtain a reasonable S/N. It should be noted that the measurement of functionally important data from model membranes containing membrane active peptides demands the use of a very low peptide concentration



**Figure 7.** 2D <sup>1</sup>H/<sup>15</sup>N HSQC experiments with a 100 ms recycle delay provided the optimum S/N. 2D HSQC spectra of  $q = 0.5$  isotropic bicelles containing a 9.3 mM unlabeled MSI-78 and a 2.96 mM copper-chelated lipid obtained by setting the total data collection time to 53 min with (A) 100 ms and (B) 600 ms recycle delays. (C) A comparison of the signal-to-noise ratio against recycle delay for selected peaks from 2D HSQC spectra; since the S/N observed for longer recycle delays was poor as seen in (B), only those peaks that had reasonable S/N are included in this plot. Other experimental and data processing details are as mentioned in the Figure 5 caption.



**Figure 8.** Isotropic bicelles with  $q = 0.25$  provided higher resolution and sensitivity than that of  $q = 0.5$ . (A) A comparison of 2D SOFAST-<sup>1</sup>H/<sup>15</sup>N-HMQC experiments of 9.3 mM unlabeled MSI-78 reconstituted in  $q = 0.5$  (dark) and  $q = 0.25$  (red) isotropic bicelles with a 2.96 mM Cu-DMPE-DTPA. Each 2D HSQC spectrum was obtained from 64  $t_1$  experiments and 256 scans. Other experimental and data processing details are as mentioned in Figure 5 caption. Signal-to-noise ratio (B), 1D <sup>1</sup>H chemical shift projections (C), and full-width at half-maximum (FWHM) values (D) obtained from 2D SOFAST-HMQC spectra are compared.

that makes NMR measurements to be difficult.<sup>3</sup> This becomes even more challenging when isotopically labeled peptides are unavailable; this is unfortunately the case for a significant number of interesting systems such as mammalian membrane proteins and small peptides that lyse bacteria. It has been proven to be expensive to biologically obtain most membrane-associated peptides

and proteins. Therefore, structural studies on such systems are often restricted to unlabeled peptides or site-specifically labeled peptides. On the other hand, results presented in this study demonstrate the feasibility of NMR structural studies at natural-abundance of membrane-associated peptides and therefore could be useful to overcome the above-mentioned challenges. Therefore,

we believe that this successful demonstration opens up avenues to investigate the structural, dynamical, and aggregation properties of functional biomolecules like amyloid proteins and antimicrobial peptides.

To check the effect of the size of isotropic bicelles, full-width at half-maximum (FWHM) of peaks from 2D  $^1\text{H}/^{15}\text{N}$  SOFAST-HMQC spectra of unlabeled-MSI-78 embedded in isotropic bicelles with different  $q$  ratios were measured. As shown in Figure 8, the measured S/N and FWHM values from isotropic bicelles with  $q$  ratios 0.25 and  $q = 0.5$  are comparable. These results suggest that the paramagnetic effect rendered by the copper-chelated lipid is independent of the size of the bicelle. Therefore, the large isotropic bicelles with  $q = 0.5$  that contain more lipids and hence a larger planar bilayer surface can be used in structural studies using solution NMR spectroscopy.

## CONCLUSIONS

In this study, we have demonstrated the use of a copper-chelated lipid to shorten the  $T_1$  of protons for fast data acquisition from an unlabeled antimicrobial peptide embedded in isotropic bicelles. We note that previous studies have reported NMR spectra of short peptides at natural-abundance but these studies used either high concentration of a water-soluble protein or long data acquisition and therefore such studies have not been common.<sup>58,59</sup> Several solution and solid-state NMR studies on the successful use of paramagnetic salts to shorten the proton  $T_1$  value of water-soluble systems have been previously reported.<sup>30,33,34,42</sup> The high concentration of paramagnetic salts (for example  $\sim 30$ – $50$  mM concentration) used in these studies are not suitable for membrane systems as the mobile paramagnetic ions are ineffective in shortening  $T_1$  value of membrane embedded molecules and they could also result in RF heating and power lossy effects.<sup>24–33</sup> On the other hand, our approach effectively reduces the required concentration of the ion required to speed-up the  $T_1$  process, and also renders the preparation of bicelles without altering their properties. The 4 times faster data acquisition than the SOFAST-based NMR experiments could be valuable to study various biochemical processes. Though line-narrowing instead of line-broadening due to the presence of  $\text{Cu}^{2+}$  was observed in our study (Figure 6B), a systematic analysis of the changes in  $T_2$  values could be useful to determine the topology of a membrane protein as demonstrated in a previous study using Gd(DOTA).<sup>42</sup> We believe that a comparative study on the paramagnetic effect of different metals like  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Gd}^{3+}$ ,  $\text{Dy}^{3+}$  and  $\text{Cu}^{2+}$  chelated to the DMPE lipid could be valuable for future structural studies on membrane proteins.

## ASSOCIATED CONTENT

**S Supporting Information.**  $R_1$  data of isotropic bicelles and optimization of the recycle delay in the presence of paramagnetic effect. These materials are available free of charge via the Internet at <http://pubs.acs.org>.

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## ACKNOWLEDGMENT

This research was supported by NIH (GM084018 and GM095640 to A.R.).

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