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

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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}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 (~3 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.

Keywords

NMR spectroscopy; sensitivity enhancement; membrane proteins; bicelles

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.^{1–9} However, in spite of the recent technical advances, relatively poor sensitivity of NMR spectroscopy continues to be a major bottleneck for high-throughput applications.^{10–13} 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.^{14–16} The mandatory requirement for isotopic labeling of membrane proteins further limits NMR applications, as there are numerous molecules that

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Supporting Information Available: *R₂* data of isotropic bicelles and optimization of the recycle delay in the presence of paramagnetic effect are given in the supporting information. These materials are available free of charge via the internet at <http://pubs.acs.org>.

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-78¹⁷, Figure 1) at natural-abundance of ¹⁵N nuclei and the data can be collected four times faster, even when the SOFAST-HMQC¹⁸ 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* = [DMPC]/[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.¹⁹ 3D structure of dimeric form of MSI-78 embedded in DPC micelles (Figure 5B) determined from NMR experiments has been reported.²⁰ As shown in Figure 1, MSI-78 was designed to exhibit an amphipathic α -helical structure between residues 4 and 19 (highlighted in red) in a membrane environment whereas it is unstructured in solution.¹⁹ Solid-state NMR studies reported the lipid-peptide interactions, membrane-associated structure, and its mechanism of membrane disruption of MSI-78.^{21–22}

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 in solid-state samples.^{13,23–32} Solution NMR studies focused on water-soluble proteins while solid-state NMR studies utilized paramagnetic salts on crystalline biomolecules. T_1 of ¹³C nuclei was shortened in carbon-detected solution NMR experiments using Gd³⁺.³³ Later, Wuthrich and coworkers 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 s to 0.3 s using 1 mM Gd³⁺.³⁴ Since the line broadening effects due to Gd³⁺'s long (nano to micro seconds) electronic relaxation times (T_{1e} and T_{2e}) are not suitable for proton-detected experiments, Ni²⁺ that has a shorter relaxation time (pico to nano seconds)³⁵ was used to shorten the T_1 of protons from macromolecules and water without a line broadening effect.³⁰ Recently, Ishii and coworkers have demonstrated the use of Cu²⁺ 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.^{25,26} Reif and coworkers have demonstrated further signal enhancement due to T_1 shortening by using Cu²⁺ paramagnetic effect in deuterated proteins in which labile protons are back-exchanged from H₂O/D₂O.²⁹ A recent solid-state MAS NMR study by Jaroniec and coworkers have shown rapid data collection by covalently bound paramagnetic tags in crystalline proteins.³¹ Other solution^{36–42} and solid-state NMR^{43,44} 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.²⁵ 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^{24–32}; (ii) it can be mixed homogeneously without altering the properties of other components of bicelles; (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^{45–51} were prepared using the following procedure. In the case of $q=0.5$ bicelles, 55.0 mg of DMPC and 73.6 mg of DHPC were cosolubilized in 1 ml of chloroform. Subsequently, chloroform was removed under a stream of 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 μ 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 minutes, and subjected to more than 4 freeze/heat cycles between liquid nitrogen and 40 °C of water. Once a satisfactory mixing of the components was completed, the sample (~330 μ l) was transferred to a 5 mm Shigemi NMR glass tube. The isotropic bicelle formation was examined using ^{31}P NMR experiments after 30 minutes of incubation at 37 °C in the NMR magnet.

Isotropic bicelles 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 bicelles containing the copper-chelated lipid, 0.349 mg of $Cu(NO_3)_2 \cdot 2.5H_2O$ was added (for q ratio = 0.5 case) to a mixture of isotropic bicelles, 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:Cu-DMPE-DTPA was 53.91:1 while DMPE-DTPA: $Cu(NO_3)_2 \cdot 2.5H_2O$ 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 (T_1) relaxation of protons from bicelles for various concentrations of copper-chelated DMPE (Figures 3, 4, 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^{52–57} makes it a better model membrane, we chose to use bicelles to investigate the paramagnetic effect of Cu^{2+} for fast NMR data acquisition. Isotropic bicelles composed of DMPC, DHPC, and MSI-78 were used in NMR experiments. Since free Cu^{2+} ions could interact with the protein and also could result in RF-induced heating, a Cu^{2+} -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, ^1H spin-inversion-recovery experiments were performed. High-resolution ^1H 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^{2+} -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 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 ^1H (Figure 4) and ^{31}P (data not included) chemical shift spectra of bicelles with and without the Cu^{2+} -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 bicelles for different concentrations of Cu^{2+} -DMPE-DTPA lipid were measured to evaluate the line broadening effect (Figure 4). Our results suggest that the presence of Cu^{2+} -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 $^1\text{H}/^{15}\text{N}$ SOFAST-HMQC experiments on bicelles containing an unlabeled MSI-78 was compared for various concentrations of Cu^{2+} -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^{2+} 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^{2+} was sufficient to increase the S/N by 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^{2+} -chelated DMPE. Interestingly, the use of a 2.96 mM Cu^{2+} -chelated DMPE provided the highest sensitivity (Figures 6A and 6C) and further increase in the concentration of Cu^{2+} -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^{2+} -chelated DMPE (Figure 3).

Line widths of resonances observed in 2D $^1\text{H}/^{15}\text{N}$ 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^{2+} -DMPE-DTPA lipid; slightly narrower peaks were observed for most sites of MSI-78 in the presence of 2.96 mM Cu^{2+} -DMPE-DTPA lipid. On the other hand, the use of higher concentrations (5.89 and 11.7 mM) of Cu^{2+} -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^{2+} ions. Since a concentration <3 mM Cu^{2+} -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^{2+} ions is not a major concern for this study. However, a systematic analysis of changes in the T_2 values due to the presence of Cu^{2+} -DMPE-DTPA could provide valuable information on the topology of a membrane protein as previously demonstrated for OMPX using Gd(DOTA).⁴² Details on the paramagnetic effects of different types of ions on T_1 and T_2 can be found in a recent review article.^{32,37}

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 $^1\text{H}/^{15}\text{N}$ HSQC experiments were performed on bicelles containing MSI-78 with no ^{15}N labeling to measure the paramagnetic ion induced sensitivity gain for various concentrations of Cu^{2+} -chelated DMPE. Remarkably, bicelles containing the Cu^{2+} -chelated DMPE provided HSQC spectra with a reasonable S/N within 2 hours 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 Figures 7C and 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 that makes NMR measurements to be difficult.³ 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.

In order 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.^{58,59} 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.^{30,33,34,42} The high concentration of paramagnetic salts (for example ~30 to 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.^{24–33} 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 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 $\text{Gd}(\text{DOTA})$.⁴² We believe that a comparative study on the paramagnetic effect of different metals like Ni^{2+} , Co^{2+} , Gd^{3+} , Dy^{3+} and Cu^{2+} chelated to the DMPE lipid could be valuable for future structural studies on membrane proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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

Figure 2.

(A) The 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.

Figure 3.

Spin-lattice relaxation rate (R_1) of protons from DMPC/DHPC isotropic bicelles ($q=0.5$) for different concentrations of a copper-chelated lipid, Cu^{2+} -DMPE-DTPA at 35 °C. The R_1 values were determined from ^1H -spin-inversion recovery experiments and the estimated error from the best-fitting of experimental data is about ± 0.05 . One-dimensional ^1H chemical shift spectrum of DMPC/DHPC isotropic bicelles with 0 mM Cu^{2+} -DMPE-DTPA at 35 °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 ^1H NMR spectra of DMPC/DHPC isotropic bicelles ($q = 0.5$) for different concentrations of a copper-chelated lipid, Cu^{2+} -DMPE-DTPA, at 35 °C. The close resemblance of proton chemical shift spectra for different Cu^{2+} -DMPE-DTPA concentrations suggests that there is no change in the bicellar properties of the samples due to the presence of Cu^{2+} ions. All measurements were carried out on a 400 MHz Varian solid-state NMR spectrometer.

Figure 5. A four-fold increase in the sensitivity of 2D SOFAST-HMQC experiments

(A) 2D SOFAST-¹H/¹⁵N-HMQC¹⁸ 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.²⁰ Solid-state NMR studies have shown the membrane-surface association of this peptide and its ability to function by forming toroidal pores.²² The effect of a Cu²⁺-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 ¹H NMR experiments (Figures 3 and 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 ¹H/¹⁵N SOFAST-HMQC spectra and are compared in Figures 6. Further increase in the concentration of Cu²⁺ either did not increase the S/N or resulted in undesirable effects like line broadening (Figure 6). (D) 1D ¹H chemical shift projection spectrum obtained from 2D SOFAST-¹H/¹⁵N-HMQC spectra that were obtained with no copper (black) and a 2.96 mM Cu²⁺-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.

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 Cu²⁺-DMPE-DTPA (black), and with a 2.96 mM Cu²⁺-DMPE-DTPA (red), 5.89 mM Cu²⁺-DMPE-DTPA (blue) and 11.7 mM Cu²⁺-DMPE-DTPA (green) at 35 °C. (C) One-dimensional ¹H chemical shift projections from 2D SOFAST-HMQC spectra. Other experimental and data processing details are as mentioned in the Figure 5 caption.

Figure 7. 2D $^1\text{H}/^{15}\text{N}$ 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- $^1\text{H}/^{15}\text{N}$ -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 ^1H chemical shift projections (C), and full-width at half-maximum (FWHM) values (D) obtained from 2D SOFAST-HMQC spectra are compared.