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Probing Residue-Specific Water–Protein Interactions in Oriented Lipid Membranes via Solid-State NMR Spectroscopy

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Abstract

Water plays a central role in membrane protein folding and function. It not only catalyzes lipid membrane self-assembly but also affects the structural integrity and conformational dynamics of membrane proteins. Magic angle spinning (MAS) solid-state NMR (ssNMR) is the technique of choice for measuring water accessibility of membrane proteins, providing a measure for membrane protein topology and insertion within lipid bilayers. However, the sensitivity and resolution of membrane protein samples for MAS experiments are often dictated by hydration levels, which affect the structural dynamics of membrane proteins. Oriented-sample ssNMR (OS-ssNMR) is a complementary technique to determine both structure and topology of membrane proteins in liquid crystalline bilayers. Recent advancements in OS-ssNMR involve the use of oriented bicellar phases that have improved both sensitivity and resolution. Importantly, for bicelle formation and orientation, lipid bilayers must be well organized and hydrated, resulting in the protein's topology being similar to that found in native membranes. Under these conditions, the NMR resonances become relatively narrow, enabling a better separation of ¹H–¹⁵N dipolar couplings and anisotropic ¹⁵N chemical shifts with separated local field (SLF) experiments. Here, we report a residue-specific water accessibility experiment for a small membrane protein, sarcolipin (SLN), embedded in oriented lipid bicelles as probed by new water-edited SLF (WE-SLF) experiments. We show that SLN's residues belonging to the juxtamembrane region are more exposed to the water–lipid interface than the corresponding membrane-embedded residues. The information that can be obtained from the WE-SLF experiments can be interpreted using a simple theoretical model based on spin-diffusion theory and offers a complete characterization of membrane proteins in realistic membrane bilayer systems.

Graphical abstract

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Introduction

Water–protein interactions play an important role in driving the structural dynamics and functional properties of proteins.^{1–5} Hydration forces mediate protein's packing via hydrogen bond networks and shape the structural integrity.^{6,7} For membrane proteins, water has a dual role: it maintains the functional integrity of the domains that are more solvent-exposed (juxtamembrane and globular domains of membrane proteins) and plays an essential role in the structure and function of lipid membranes. In fact, in the absence of water, lipid membranes would not exist as their basic structural units self-assemble due to mainly hydrophobic effects.⁸

NMR is the only atomic resolution technique that can be used to study water–protein interactions in fully hydrated samples. Although NMR cannot visualize bound water molecules in active sites like X-ray crystallography, it can determine the relative exposure of atomic groups (e.g., backbone amides), monitoring either hydrogen/deuterium exchange⁹ or using tailored pulse sequences.^{10–13} Recently, these methodologies have been extended to magic angle spinning (MAS) solid-state NMR (ssNMR) of biological macromolecules.^{14–16} These studies have demonstrated that the water–protein interactions in ssNMR can be probed by $^1\text{H}-^1\text{H}$ polarization transfer via chemical exchange, nuclear Overhauser effect (NOE), or dipolar spin diffusion between water and the surface of the protein.¹⁷ However, high sensitivity and resolution of MAS samples often require low levels of hydration¹⁸ with high protein:lipid ratios. Ideal hydration levels for MAS samples were found to be between 30 and 40%, as determined for the integral membrane protein diacylglycerol kinase (DAGK).¹⁸ Nonetheless, the critical hydration level required for protein function may change from system to system, and low hydration levels are likely to affect both the structural dynamics and function of membrane-embedded enzymes.^{8,19}

A synergistic ssNMR approach is oriented-sample ssNMR (OS-ssNMR), which provides direct topological information of membrane proteins in more realistic membrane models.^{20–22} In fact, the formation of lipid phases, either mechanically or magnetically oriented, requires high hydration levels to reach liquid crystalline lipid phases.²⁰ These systems are suitable for characterizing water–protein interactions under native-like conditions.^{23–27} Currently, the water–protein interactions of oriented membrane proteins require the comparison of the separated local field (SLF) spectra of protein samples reconstituted by

using protonated and deuterated buffer conditions.^{28,29} In this approach, the residues exchanging with deuterated water cannot be detected in the SLF spectrum and are flagged as water-exposed.^{28,29} A drawback of this method is the preparation of two separate samples and/or the manipulation of the sample to exchange protium with deuterium.

In this work, we propose a spectral-editing technique for oriented membrane proteins to map the residue-specific water–protein interactions in oriented samples. We designed a water–edited (WE) SLF experiment that enables us to determine residue-specific exposure of the amide groups in oriented lipid membranes. In principle, this new approach can be applied to both mechanically and magnetically aligned samples. However, the more favorable spectroscopic properties of lipid bicelles^{30,31} coupled with the sensitivity-enhanced (SE) method^{32,33} make this approach much more powerful. The experiment is designed as a pseudo-three-dimensional (3D) experiment to obtain a protein fingerprint (polarity index slant angle wheel)³⁴ as a function of mixing time and determine the water exposure for each resolved site. As a benchmark, we used uniformly ¹⁵N-labeled sarcolipin (SLN), a single-pass membrane protein, whose structure has been fully characterized by solution NMR in micelles^{35,36} and in lipid membranes using both MAS and oriented ssNMR.^{37,38} SLN represents a prototypical transmembrane domain, with a membrane-embedded hydrophobic domain (domain II), a juxtamembrane region (domain Ib), and two hydrophilic termini comprising basic and aromatic residues that anchor SLN in the lipid membrane. H/D exchange factor measurements³⁹ using solution NMR studies indicate that both N- and C-termini and the juxtamembrane region (domain Ib) are water-exposed, whereas domain II is buried in the membrane bilayer and is virtually inaccessible to water. The WE-SLF data agree with the exchange factor data and can be interpreted with a simple theoretical model based on spin-diffusion theory,⁴⁰ providing a new, general approach to test water accessibility for oriented NMR samples.

Material and Methods

Sample Preparation

Uniformly ¹⁵N-labeled SLN was expressed as a fusion protein with maltose-binding protein (MBP) in *Escherichia coli*, as previously described.^{41,42} SLN was purified via amylose affinity chromatography, and MBP was cleaved using tobacco etch virus protease. The cleaved proteins were dialyzed in water, and the SLN precipitate was further purified using reverse-phase high-performance liquid chromatography. The purified SLN was lyophilized overnight. The lipids, 31 mg deuterated 1,2-dimyristoyl-*sn*-glycero-3-phospho-choline (DMPC) and 6.7 mg deuterated 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (D6PC), were dried and lyophilized overnight. The lyophilized SLN (1.9 mg) was co-dissolved with the deuterated DMPC using 500 μ L of 1:1 ratio of methanol and water. The methanol was evaporated under a stream of N₂ gas, and the mixture was lyophilized overnight to ensure removal of any remaining trace of organic solvent. The SLN/ DMPC mixture was resuspended in 70 μ L NMR buffer (20 mM *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid, 50 mM KCl, 2.5 mM MgCl₂, 0.02% NaN₃) and sonicated. D6PC dissolved in 50 μ L NMR buffer was then added. To prepare bicelles that align with the membrane normal parallel to the direction of the static field, YbCl₃ was added to a final concentration of 5

mM. Bicelles were formed after 3–5 freeze/ thaw cycles, which resulted in a nonviscous solution between 0 and 15 °C and a viscous and clear solution above 30 °C. The final volume was adjusted to 160 μL by addition of NMR buffer, giving a final lipid concentration of 28% (w/v). Bicelles were transferred to a flat-bottom glass tube (New Era Enterprises) and tightly sealed with a polytetrafluoroethylene cap.

NMR Spectroscopy

All of the NMR experiments were performed on a Varian NMR System spectrometer operating at a ^1H frequency of 700 MHz equipped with a low-E bicelle probe built by the RF program at the National High Magnetic Field Laboratory in Florida.⁴³ A 90° pulse of 5 μs that corresponds to 50 kHz was used for both ^{15}N and ^1H channels, and a cross-polarization (CP) time of 500 μs was applied at ^1H and ^{15}N radio frequency (RF) field strengths of ~ 50 kHz. For WE experiments, the τ value was set to 1.8 ms that acts as a T_2 filter and dephases ^1H magnetization of the protein and lipid molecules, while retaining the water magnetization. Water–protein polarization transfer was achieved by varying the τ_{mix} value. For heteronuclear correlation (HETCOR) and SE-HETCOR experiments,⁴⁴ the windowless isotropic mixing (WIM) pulse sequence⁴⁵ was used for heteronuclear polarization transfer. During SE-polarization inversion spin exchange at the magic angle (PISEMA)³³, the t_1 frequency-switched Lee–Goldburg (FSLG)⁴⁶ homonuclear decoupling was applied on the proton channel with a 50 kHz effective field,⁴⁷ whereas phase-switched spinlock pulses on the ^{15}N channel were Hartmann–Hahn⁴⁸ matched with a 50 kHz RF amplitude during t_1 evolution. For sensitivity enhancement, the τ delay was set to 75 μs , which corresponds to three cycles of FSLG with an effective RF amplitude of 80 kHz. Small phase incremental alteration 64 (SPINAL64) decoupling was used during acquisition with 50 kHz ^1H RF amplitude.⁴⁹ All of the data were processed using NMRPipe⁵⁰ and analyzed using Sparky.⁵¹

Theory and Analysis of ssNMR Data

To interpret the spin diffusion in our system, we started from the general treatment of the diffusion of z magnetization $M(\vec{r} \rightarrow, t_m)^{40}$ at time τ_{mix}

$$\frac{\partial M(\vec{r}, \tau_{\text{mix}})}{\partial \tau_{\text{mix}}} = \vec{\nabla} \cdot \left\{ D(\vec{r}) \vec{\nabla} M(\vec{r}, \tau_{\text{mix}}) \right\} \quad (1)$$

where $D(\vec{r})$ stands for diffusion coefficients. For a two-phase system, the magnetization is initially homogeneously distributed in phase A (water) and zero in phase B (protein). As the magnetization transfers from phase A to B, the buildup of exchange intensity I_B can be simplified as follows

$$\frac{I_B(\tau_{\text{mix}})}{I_B(\tau_{\text{mix}} \rightarrow \infty)} = \sqrt{\frac{D\tau_{\text{mix}}}{\pi}} \frac{1}{f_A f_B} \frac{S_{AB}^{\text{tot}}}{V_{\text{tot}}} - O(\sqrt{\tau_{\text{mix}}^2}) \quad (2)$$

in which f_A and f_B represent the volume fractions of phases A and B, respectively. S_{AB}^{tot} denotes the total area of all interfaces between phases A and B, and V^{tot} stands for the total sample volume. Assuming $f_A \approx 1$, and neglecting the quadratic terms $O(\sqrt{\tau_{\text{mix}}^2})$, we obtain

$$\frac{I_B(\tau_{\text{mix}})}{I_B(\tau_{\text{mix}} \rightarrow \infty)} \approx \sqrt{\frac{D\tau_{\text{mix}}}{\pi}} \frac{S_{AB}^{\text{tot}}}{V_B} \quad (3)$$

in which V_B denotes the volume of phase B. The magnetization transfer between water and a membrane-embedded protein results in a slow spin diffusion at the water–protein interface (WP) and a much faster intramolecular spin diffusion within the protein (PP). Therefore, we use an effective diffusion coefficient D_{eff} to account for the observed magnetization transfer in this more complex system

$$\frac{M_P(\tau_{\text{mix}})}{M_P(\tau_{\text{mix}} \rightarrow \infty)} \approx \sqrt{\frac{D_{\text{eff}}}{\pi}} \frac{S_{WP}}{V_P} \sqrt{\tau_{\text{mix}}} \quad (4)$$

where S_{WP} stands for the total area of the water–protein interface, and V_P is the volume of protein. For $M_P(\tau_{\text{mix}}^S) = M_P(\tau_{\text{mix}} \rightarrow \infty)$, we obtain

$$\sqrt{\tau_{\text{mix}}^S} = \sqrt{\frac{\pi}{D_{\text{eff}}}} \frac{V_P}{S_{WP}} \quad (5)$$

Therefore, the magnetization of each residue along the protein sequence goes to saturation with different values of $\sqrt{\tau_{\text{mix}}^S}$ depending on their water exposure. Specifically, the TM domain of SLN can be approximated as a cylinder of volume $V_P = \pi R_{\text{TM}}^2 h$, where R_{TM} and h are the radius and height of the TM domain, respectively. Therefore, the residue-specific $\sqrt{\tau_{\text{mix}}^S}$ becomes linearly dependent on the position of each residue with respect to the helix

$$\sqrt{\tau_{\text{mix}}^S} \sim \sqrt{\frac{\pi}{D_{\text{eff}}}} \frac{\pi R_{\text{TM}}^2 h}{S_{WP}} \quad (6)$$

Results

The new SLF pulse sequences used to probe the water accessibility for SLN are depicted in Figure 1. Figure 1A shows the one-dimensional (1D) WE-CP experiment that uses water as

a source of polarization for recording ^{15}N signals from SLN's amide backbone. The pulse sequence has an initial water-filter element identical to the MAS experiments.^{14,15,17} An initial 90° pulse is followed by a spin-echo (~ 2 ms) that serves as a T_2 filter to dephase the protein as well as the lipid magnetization. The mobile water molecules, with longer T_2 relaxation, retain their magnetization, which is then flipped to the z-direction by another 90° pulse. A subsequent longitudinal mixing period (τ_{mix}) enables the transfer of ^1H polarization from water to protein, which is then transferred to ^{15}N using a 90° pulse followed by Hartmann–Hahn CP.⁴⁸ During CP, only the immobilized residues are subjected to heteronuclear polarization transfer via dipolar couplings. Figure 1B shows the implementation of the T_2 water-filter HETCOR experiment, WE-HETCOR. In this case, the t_1 evolution before τ_{mix} encodes the ^1H chemical shift of water. After the ^1H – ^1H transfer, the amide proton magnetization is selectively transferred to the corresponding amide ^{15}N by the WIM24-CP⁴⁵ sequence followed by t_2 acquisition under SPINAL heteronuclear decoupling.⁴⁹ Figure 1C shows the two-dimensional (2D) WESE-PISEMA experiment, where the initial τ period, preceded by a 90° pulse, retains ^1H polarization from water, while dephasing the ^1H coherences arising from the protein and lipids. The protons of the protein are polarized via homonuclear zz transfer during the τ_{mix} period followed by a SE-PISEMA sequence.

The application of WE-CP to a $\text{U-}^{15}\text{N}$ SLN sample reconstituted in lipid bicelles is shown in Figure 2A at different ^1H – ^1H mixing times. Overall, the buildup is similar to that observed for the MAS experiments, with a sigmoidal trend.^{14,15,17} At zero mixing time (Figure 1A), no signal is detected, confirming that the protein magnetization is completely dephased due to the initial spin-echo period. At short mixing times, we observe only a few selected signals. As the mixing time increases, the intensity of several other peaks builds up and reaches saturation at approximately 100 ms (Figure 2B). A comparison between 2D SE-HETCOR and WE-HETCOR experiments is reported in Figure 2C. A ^1H – ^{15}N correlation spectrum obtained with the SE-HETCOR experiment in the absence of the T_2 filter is shown in red. The spectrum acquired using the WE-HETCOR experiment with a 1.8 ms T_2 filter and 100 ms mixing time (τ_{mix}) is reported in black. As expected, the ^{15}N resonances of SLN's backbone are correlated with the water resonance at 4.7 ppm in the indirect dimension, whereas no correlations are obtained with amide or lipid protons, demonstrating that the T_2 filter retains only water magnetization.

To obtain site-specific water–protein interactions for the $\text{U-}^{15}\text{N}$ SLN sample in lipid bicelles, we incorporated the T_2 filter into the SE-PISEMA experiment. SLN is a 31-residue helical membrane protein with short N- and C-terminal tails and is anchored to the lipid membrane via positively charged arginines.^{35,38,52} MAS and oriented ssNMR experiments carried out in our laboratory have revealed the high-resolution structural topology of SLN in lipid bicelles.³⁷ The structure of SLN consists of a regular α -helix, flanked by several aromatic amino acids on both the luminal and cytoplasmic sides of the membrane bilayer. The SE-PISEMA spectra are well-resolved and enabled residue-specific assignments using both 2D and 3D experiments.³⁷ Figure 3 shows the SE-PISEMA and WESE-PISEMA spectra of SLN, where τ_{mix} was set to 100 ms for ^1H – ^1H transfer. As expected, at 100 ms mixing time, almost all of the residues are polarized by water via the spin-diffusion mechanism. Using a WESE-PISEMA sequence coded as a pseudo-3D experiment, we

monitored the spin-diffusion kinetics, with τ_{mix} values spanning from 1 to 100 ms. Figure 4 shows the resultant spectra at various mixing times. At short mixing times, the WESE-PISEMA spectra display very low signal-to-noise ratios. For this reason, the 2D spectra with mixing times less than 10 ms were acquired with 512 scans, whereas 96 scans were sufficient for spectra acquired at longer mixing times (Figure 4). For comparison, all of the spectra were plotted at the same noise level, and peaks corresponding to a signal-to-noise ratio greater than eight were picked. To interpret the spin diffusion of the z magnetization, we utilized the spin-diffusion theory described by Schmidt-Rohr and Spiess.⁴⁰ Assuming a simple geometric model for the single transmembrane domain of SLN, we modified the master equation to obtain eq 5 (see Theory and Analysis of ssNMR Data). The buildup plots of the intensities of SLN residues as a function of the square root of mixing time are shown in Figure 5. Note that due to dynamics, both N- and C-terminal residues are not sensitive to CP-based experiments and are not visible in the PISEMA spectra.⁵³ As for the integrated 1D spectrum of the WE-CP and MAS WE experiments,¹⁷ the individual curves follow a sigmoidal behavior. The initial rate of polarization, 1–7 ms, is relatively fast for residues in domain Ib, including N11, T13, I14, and V15. Based on our model, this depends on the fast kinetics of polarization transfer from water to protein. This pattern is in agreement with previous solution NMR data carried out on SLN reconstituted in dodecylphosphocholine micelles, showing that the juxtamembrane domain Ib of SLN (residues 7–14) is more dynamic and has a higher solvent accessibility.³⁶ Note that the CP transfer is also influenced by protein dynamics and mosaic spread, as reflected by the nonuniform intensities of the peaks.⁵⁴ Because of its lower sensitivity, N11 could only be detected at 7 ms mixing time. In addition, residues T13 and L24 are overlapped in the 2D SE-PISEMA spectrum. To resolve this ambiguity, we ran a WE-CP experiment on a selectively leucine-labeled sample using 3 ms mixing time (Figure 6). None of the leucine peaks were detected in the WE-CP spectrum. The latter confirms that leucine residues in domain II of SLN are embedded within the core of the membrane and are not water-accessible. Nonetheless, these residues show clear peaks at longer mixing times, showing that spin diffusion is dominant and all of the residues can be polarized. On the basis of eq 6 in the previous section, the residue-specific mixing time $\sqrt{\tau_{\text{mix}}^S}$ was fitted against the height of residue in the TM regions. Overall, we found a linear relationship, where most of the residues lie within the 99% confidence intervals, supporting the theoretical model (Figure 7). The small deviations from the theoretical model found for residues L16 and I17 are due to the low signal-to-noise ratio that is unavoidable for these sample preparations. Figure 8 summarizes the site-specific water accessibility for SLN as obtained from the WE experiments. The backbone sites that are dynamic and not visible in the WESE-PISEMA spectra are represented in red. The other sites with increasing protection from the solvent are reported in yellow, green, cyan, and blue. The presence of resonances for the most protected residues in the PISEMA experiments at longer mixing times is due to water $\rightarrow$ protein $\rightarrow$ protein spin diffusion, which reaches complete saturation at mixing times greater than 100 ms. Overall, there is very good agreement between the exchange factors measured for SLN by solution NMR in micelles³⁶ and WESE-PISEMA spectra in lipid bicelles.

Discussion

For soluble proteins, water accessibility provides important insights into structural stability and dynamics.⁵⁵ On the other hand, for membrane proteins, it also provides a ruler to establish their insertion and topology within lipid membranes. In spite of the significant progress made by hydrogen/ deuterium mass spectrometry,⁵⁶ NMR remains the technique of choice to determine the relative water accessibility of protein residues at atomic resolution. Although solution NMR is able to map both kinetics and thermodynamics of membrane proteins in micelles, there is mounting evidence that micelles do not mimic membrane environments.^{23,57,58} Both MAS and OS-ssNMR utilize lipid environments to reconstitute membrane protein samples. Unlike in MAS, the samples used in OS-ssNMR contain more than 70% water; hence, this method is ideal for probing water–protein interactions in a residue-specific manner. In particular, bicelles are emerging as a robust membrane-mimetic system for both small and large membrane proteins.^{30,59} The self-assembly of lipid molecules for bicelle formation requires high hydration levels to form oriented phases.⁶⁰ A significant advantage of bicelles is the long relaxation in the dipolar dimension of the SLF experiments that enable the implementation of sensitivity-enhancement schemes⁶¹ and boost the sensitivity of the static NMR experiments. The new experiments reported here make it possible to monitor the water exposure of amide resonances with relatively high sensitivity. As for the previous MAS experiment, water–protein polarization transfer is mediated by chemical exchange, NOE, and spin-diffusion mechanisms.^{2,17} These studies have demonstrated that, although the rate of ^1H – ^1H transfer depends on these mechanisms, the shorter mixing times used make it possible to select the residues most accessible to water. The behavior of the polarization transfer in our static WE experiments follows the same trend and could also be utilized as a spectral-editing technique.^{62,63} From an experimental point of view, a key parameter is the mixing time, τ_{mix} , which needs to be optimized for polarization transfer from water to protein. For SLN, we found that even at short mixing times (1–3 ms), the residues of the juxtamembrane domain Ib are polarized, indicating the close proximity of water molecules.

Although there have been several advancements in the field of OS-ssNMR,²² there are still limitations for larger membrane proteins that present significant overlap of resonances in the SLF experiments. WE experiments under MAS conditions have been successfully used for studying G protein-coupled receptor, potassium channel, and M2 channel membrane proteins,¹⁴ but the spectral simplification via the WE experiments presented in this article could be a turning point for new methodological developments to determine the 3D structure of larger, oriented membrane proteins.

Conclusions

We demonstrated the ability of OS-ssNMR to investigate residue-specific hydration of membrane proteins reconstituted in oriented lipid bicelles. The relative exposure of the residues to water provides a ruler to establish the location and the structural topology of the transmembrane segments in fully hydrated liquid crystalline membranes. Indeed, these results demonstrate that the water molecules are indeed an intrinsic part of the membrane protein environment and can be further exploited to study protein function. In the case of

SLN, the accessibility of the juxtamembrane domain Ib supports the plasticity of this protein and the importance of this domain in the regulation of the Ca^{2+} -ATPase, supporting previous studies carried out in detergent micelles. Together with the recent advancements in sample preparation and NMR methodology, the WE experiments presented here will bring a new dimension in OS-ssNMR to study membrane proteins.

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Figure 1. WE-oriented ssNMR. Pulse sequences for (A) WE CP, (B) 2D HETCOR experiment for water–protein correlation, and (C) WESE-PISEMA that can be used as pseudo-3D by varying the τ_{mix} value.

Figure 2.

(A) WE-CP spectra obtained at various mixing times using the pulse sequence of Figure 1A. (B) Integrated intensity of the 1D spectra between 130 and 210 ppm is plotted with respect to the square root of mixing time. (C) 2D HETCOR spectrum (black) between water and protein residues obtained from the pulse sequence shown in A using 100 ms mixing time and $^1\text{H}^{\text{N}} - ^{15}\text{N}$ correlation (red) of the protein backbone obtained from the SE-HETCOR experiment.

Figure 3. 2D SE-PISEMA and WESE-PISEMA spectra of uniformly ^{15}N -labeled SLN in oriented flipped bicelles. A mixing time of 100 ms was used for WESE-PISEMA.

Figure 4.

WESE-PISEMA spectra of SLN obtained at various mixing times using the pulse sequence of Figure 1C. All of the spectra are plotted at the same noise level. The τ_{mix} values were 1, 3, 5, 7, 9, 10, 50, 70, and 100 ms. The spectra at τ_{mix} values less than 10 ms were acquired with 512 scans. The remaining spectra were acquired with 96 scans. For comparison, all of the spectra are plotted using the same noise level.

Figure 5.

Peak intensities of each residue obtained from Figure 4 plotted with respect to the square root of mixing time. The intensity of each peak was normalized relative to its SE-PISEMA intensity. The residues near the termini build up faster than those within the transmembrane region. The residues with peaks that are overlapped, such as T13, are not plotted.

Figure 6.

CP (top) and WE-CP (bottom) spectra of selectively leucine-labeled SLN. WE-CP was acquired with a mixing time of 3 ms. No peaks are observed, indicating that L24 is not accessible to water at 1–3 ms mixing time.

Figure 7.

Model of residue-specific magnetization transfer from water to SLN. (A) Schematic illustration of the membrane protein complex. SLN's helical structure can be approximated as a tilted cylinder with a radius R . The height, h , is the distance of the residue in the TM region from the water—protein interface. (B) Linear fitting (red) of the residue-specific $\sqrt{t_m^S}$ against h of each residue. The boundary of the 99% confidence intervals is shown as blue lines.

Figure 8.

Water-exposed residues highlighted on the SLN structure obtained from molecular dynamics simulations.⁵² Left: distribution of the water molecules as mapped during the molecular dynamics trajectories. Right: structure of SLN in lipid membranes. Red indicates residues that are too mobile to be observed in PISEMA but are assumed to be water-exposed. Otherwise, the residues are colored according to the mixing time (1–70 ms) at which the peak has a signal-to-noise ratio of eight or greater.