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Probing Topology and Dynamics of the Second Transmembrane Domain (M2δ) of the Acetyl Choline Receptor using Magnetically Aligned Lipid Bilayers (Bicelles) and EPR Spectroscopy

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Abstract

Characterizing membrane protein structure and dynamics in the lipid bilayer membrane is very important but experimentally challenging. EPR spectroscopy offers a unique set of techniques to investigate a membrane protein structure, dynamics, topology, and distance constraints in lipid bilayers. Previously our lab demonstrated the use of magnetically aligned phospholipid bilayers (bicelles) for probing topology and dynamics of the membrane peptide M2δ of the acetyl choline receptor (AChR) as a proof of concept. In this study, magnetically aligned phospholipid bilayers and rigid spin labels were further utilized to provide improved dynamic information and topology of M2δ peptide. Seven TOAC-labeled AChR M2δ peptides were synthesized to demonstrate the utility of a multi-labeling amino acid substitution alignment strategy. Our data revealed the helical tilts to be 11°, 17°, 9°, 17°, 16°, 11°, 9° ± 4° for residues I7TOAC, Q13TOAC, A14TOAC, V15TOAC, C16TOAC, L17TOAC, and L18TOAC, respectively. The average helical tilt of the M2δ peptide was determined to be ~13°. This study also revealed that the TOAC labels were attached to the M2δ peptide with different dynamics suggesting that the sites towards the C-terminal end are more rigid when compared to the sites towards the N-terminus. The dynamics of the TOAC labeled sites were more resolved in the aligned samples when compared to the randomly disordered samples. This study highlights the use of magnetically aligned lipid bilayer EPR technique to determine a more accurate helical tilt and more resolved local dynamics of AChR M2δ peptide.

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Conflict of Interest Statement

Keywords

AchR M26 peptide; protein topology; dynamics; magnetically aligned bicelles; EPR spectroscopy

1. Introduction

Magnetically aligned phospholipid bilayers (bicelles) have been shown to provide orientational and dynamic information about integral membrane proteins in the field of NMR spectroscopy.^{1–4} Recently similar information has been successfully obtained from low field X-band EPR using the same artificial lipid environment and nitroxide spin-probes such as the cholesterol derivative, cholestane, and fatty acid derivatives such as the doxyl-steric acids^{5, 6, 7, 8} The process was optimized to include the addition of lanthanide ions which lie on the surface of the membrane with a higher magnetic susceptibility (χ) than the bicelles themselves. In a magnetic field the lanthanides cause the lipid bicelles to flip such that the orientation of the bilayer normal is either parallel or perpendicular.^{9, 10} Using the magnetically aligned bicelles, the peptide topology of the helical M26 segment of the acetylcholine receptor protein has recently been probed.¹¹ Substituting the unnatural amino acid and spin probe, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) has been used to demonstrate the utility and biological applicability of the bicelle alignment technique in determining the topology of integral membrane proteins within a lipid bilayer.

Characterizing membrane protein structural dynamics and the orientation in a lipid bilayer membrane is a challenging but important question. NMR spectroscopy, and more recently, EPR spectroscopy, have made significant breakthroughs in this field. A large number of membrane proteins still uncharacterized inspires the study of faster and more efficient ways to determine structural dynamics and topology.

This study investigates the applicability of site specific TOAC labeling and magnetically aligned bicelles to determine the helical tilt angle of membrane proteins and peptide dynamics observed at the EPR timescale. This work illustrates data from seven TOAC AchR

M28 peptides, individually labeled on a transmembrane α -helix. As indicated in the previous work, data from several peptide substitutions provide a more accurate value of helical tilt for a peptide.¹² From the data presented in this paper, the helical tilt calculated to be 9–17° falls within $\pm 4^\circ$ of the established value.^{13,14} Additionally, by using multiple peptides an assessment can be made about the secondary structure of the peptide and speed of rotation about the long axis of the integral peptide. AchR M28 peptides I7, Q13, A14, V15, C16, L17, and L18 were synthesized with the rigid label TOAC. TOAC was chosen over the extensively studied Cys-coupled MTSL probe^{15–17} due to the rigid method of its incorporation into the peptide, which allows for better reporting of topological nuances of the AchR M28 backbone^{18, 19}. In previous studies, TOAC has additionally been shown to increase the helicity of α helical peptides,^{20–22} without the alteration of its secondary structure.²³

The nicotinic acetylcholine receptors represent the first group of membrane proteins that have slowly yielded unique structural refinement and topological analysis by such methods.¹⁴ The acetylcholine receptor protein is composed of five identical subunits containing four α -helices, labeled M1–M4.^{24, 25} Of these, the structure of the M2 pore-lining domain of the nAChR has been well characterized.¹³ It is structurally similar to numerous other transporters, receptors, channels and medically significant membrane proteins.²⁶ The M28 peptide of acetyl choline receptor forms a functioning ion-conducting pore in human erythrocyte membranes.²⁷ Despite of abundance and importance of AchR M28 peptide, detail structural and dynamic information is still lacking. This study will also provide important aspects of site specific helical tilt angle and dynamic information in a lipid bilayer. For these reasons, the M28, one subunit of the M2 domain, represents an ideal model system on which to develop rapid and more accurate methods of gauging the topology of uncharacterized membrane proteins.

2. Experimental Section

2.1 Materials

1,2-Dihexanoyl-*sn*-glycero-3-phosphatidylcholine (DHPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (PEG2000-PE), and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL). Ammonium hydroxide (aq), anisol, dysprosium(III) chloride hexahydrate (Dy³⁺), HEPES buffer, hexafluoro-2-propanol (HFIP), and thulium(III) chloride hexahydrate (Tm³⁺) were purchased from Sigma-Aldrich (St. Louis, MO). 9-fluorenylmethyloxycarbonyl-O-succinimide (Fmoc-OSu), Fmoc protected amino acids, O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU), and Fmoc-Arginine(Pbf)-NovaSyn TGA resin were purchased from Novabiochem (La Jolla, CA). 2,2,6,6-Tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) was obtained from Acros Organics (Pittsburg, PA). Acetic anhydride (Ac₂O), dichloromethane (DCM), diisopropylethylamine (DIEA), 1-hydroxybenzo-triazole (HOBt), *N*-methylpyrrolidone (NMP) and trifluoroacetic acid (TFA) were obtained from Applied Biosystems (Foster City, CA).

2.2 Synthesis and Purification

WT, I7, Q13, A14, V15, C16, L17 and L18 peptides were synthesized separately by solid phase peptide synthesis using a 433A Peptide Synthesizer from Applied Biosystems Inc. (Foster City).²⁸ Fmoc-protection chemistry was used for all of the synthesis. The peptide modeled the channel-lining of the transmembrane M26 domain of the *T. californica* nicotinic acetylcholine receptor. All amino acids purchased for this synthesis had side-chain protecting groups to decrease the probability of ancillary reactions taking place during synthesis. Fmoc-Arginine(Pbf)-NovaSyn TGA resin (substitution number = 0.22g/mmol of peptide) was used to synthesize 0.1 mmol of each peptide. Each peptide was synthesized at least twice to check the identical behavior of the peptide. The peptide sequences are provided below:

N term → C term

WT: EKMSTAISVLLAQAVCLLLTSQR

I7: EKMSTA(τ)SVLLAQAVCLLLTSQR

Q13: EKMSTAISVLLA(τ)AVCLLLTSQR

A14: EKMSTAISVLLAQ(τ)VCLLLTSQR

V15: EKMSTAISVLLAQ(τ)CLLLTSQR

C16: EKMSTAISVLLAQAV(τ)LLLSQR

L17: EKMSTAISVLLAQAVC(τ)LLTSQR

L18: EKMSTAISVLLAQAVCL(τ)LTSQR

where τ represents the TOAC position.

The Fastmoc Chemistry – 0.1 mmol protocol was provided in the SynthAssist 2.0 software from Applied Biosystems (Foster City, CA), which was customized for each synthesis. The progress of each synthesis was measured by an external UV detector, at 301nm, sensitive to the Fmoc group being removed from the N-terminus of the peptide.

Following synthesis, a mixture of TFA/TIS/anisole/H₂O (8.5%/0.5%/0.5%/0.5%) was allowed to react with each peptide for 3 hours to remove the side-chain protecting groups from the amino acids and cleave the resin from the peptide. The solution was filtered through a sintered glass filter to remove the beads of resin. Cold methyl tert-butyl ether was added to the filtrate to precipitate the peptide. Peptides were then centrifuged three times, resuspending the peptide each time, in cold methyl tert-butyl ether. Peptides were then dried with N₂ gas and placed in a vacuum desiccator overnight.

The cleaved peptides were individually purified using a C₁₈ reversed-phase semi-preparative column (218TP1010, 10 μ m, 300Å pore size, 10mm $\times$ 250 mm, column volume of 19.5mL) from Grace Vydac Inc. (Hesperia, CA), installed on an AKTA Explorer 10S HPLC from Amersham Pharmacia Biotech (Piscataway, NJ), operated by *Unicorn 3.0* software. For A14, C16 and L17, Solvent A was comprised of 0.1% TFA in H₂O and Solvent B of 0.1%

TFA + 90% Acetonitrile + 10% H₂O. V15T was purified using 0.1% TFA in H₂O as Solvent A, and 50% *n*-propanol + 30% acetonitrile + 20% H₂O + 0.1% TFA as Solvent B.¹⁴

Due to the TFA present in both the HPLC solvent and the cleavage mixture, the TOAC spin label on the peptide existed in the hydroxide form at this stage of the protocol. To regenerate the free radical, a few drops of aqueous ammonia were added to a 30% *n*-propanol + 70% H₂O solution of the peptide, and lyophilized after 3 hours. The pure regenerated peptide was stored at -20°C.

2.3 Bicelle Sample Preparation

The bicelle sample preparation was carried out following previously published literature.¹⁴ DMPC, DHPC, PEG2000PE, cholesterol and spin labeled peptide were added in a mole ratio of 3.5/1.0/0.035/0.35/0.0035 to a 25mL pear-shaped flask. The sample was dried with N₂, while being spread evenly on the walls of the flask, and then vacuum desiccated overnight. 150μL of 100mM aqueous HEPES buffer solution at a pH of 7.0 was subsequently added, to dissolve the dry sample off the walls of the flask. This was assisted by continuous vortexing, followed by sonication for 20 minutes. The sample was then freeze-thawed in liquid N₂ three times. Tm³⁺ and Dy³⁺ were added to separate aliquots of the sample to achieve a 20 mol % concentration of lanthanide with respect to DMPC.

2.4 EPR Spectroscopy

The lanthanide-doped samples were drawn into 1 mm capillary tubes from Kimax with a syringe. The ends were sealed with Critoseal (Fisher Scientific, Florence, KY). The samples were then inserted into the X-band EPR spectrometer and subjected to a temperature ramp of 21°C over 11 minutes, from 298K to 318K, in the presence of an applied magnetic field of 0.64 T. EPR experiments were conducted on a Bruker EMX X-band CW-EPR spectrometer consisting of an ER 041XG microwave bridge and an ER 4119HS cavity coupled to an ER 4131VT nitrogen gas temperature controller (temperature stability of +/− 0.2°C). EPR spectra were then collected at 318K, by taking a 42s field-swept scan (3340 center field, 100 G sweep width, 9.434 microwave frequency, 100 kHz modulation frequency, 1.0 G modulation amplitude, and a microwave power of 2 mW). EPR spectra were processed using *Igor* software (Wavemetrics, Lake Oswego, OR).

2.5 EPR Spectral Simulations

The EPR spectra were simulated using the nonlinear least-squares (NLSL) analysis program^{29, 30} using MOMD (macroscopic order, microscopic disorder) model following the similar fitting procedure published previously.¹² The principal values of hyperfine splitting tensors ($A=[5.6\text{ G}, 5.6\text{ G}, 33.3\text{ G}]$) and *g*-tensors ($g=[2.0096, 2.0064, 2.0027]$) were obtained from the literature¹² and were held constant during the fit and the rotational diffusion tensors and order parameters were determined from the best fit simulation. The data of parallel aligned samples were simulated using NLSL simulations with the introduction of the director tilt angle (Ψ). We assumed that the restoring potential is symmetric to the bilayer normal so that only the c_{20} axial symmetry term determines the order parameters. The diffusion tilt angle ($\beta_d=21^\circ$) for TOAC spin label was taken from the

literature and fixed during the simulations.³¹ The rotational correlation time is defined in terms of rotational diffusion coefficients D_R , as $\tau_c = 1/6D_R$.

3. Results

Previously, we probed the topology and dynamics of the integral membrane peptide AchR M28 inserted into magnetically aligned phospholipid bilayers (bicelles) utilizing spin-labeled EPR spectroscopy as a proof of concept.¹² In this study, we investigated the topology and dynamics of the AchR M28 peptide in more detail by analyzing magnetically aligned EPR data from seven individual TOAC labeled M28 peptide constructs: I7, Q13, A14, V15, C16, L17, and L18. Figure 1 shows sites and probes, and alignment scheme for the M28 peptide in lipid bilayers.

EPR Spectral Measurement on Aligned and Randomly Dispersed Samples

Figure 2 shows spin labeled EPR spectra of I7TOAC, Q13TOAC, A14TOAC, and V15TOAC AchR M28 peptides incorporated into DMPC/DHPC bicelles that were magnetically aligned parallel (Tm^{3+} doped sample, $n \parallel B_0$) (E, F, G and H, black traces) and randomly dispersed lipid bilayers having broader powder pattern EPR spectra (A, B, C, and D, black traces). Inspection of the EPR spectra clearly indicates that there is a significant difference in the spectral lineshape between the spectra of aligned samples and the corresponding spectra of the randomly dispersed lipid bilayers. This difference can be accounted by the orientation of the TOAC spin label with respect to magnetic field. In the case of parallel aligned samples, the z-axis of the TOAC spin label is nearly parallel with respect to the static magnetic field and leads to a large observed hyperfine splitting. In contrast, the broad powder pattern EPR spectra from the randomly dispersed samples arise due to a random distribution of TOAC labeled samples in lipid bilayers. The EPR spectra of spin labeled C16TOAC, L17TOAC, and L18TOAC AchR peptide in aligned bicelles are given in Figures 3D, 3E and 3F (black traces) and in randomly dispersed lipid bilayers are shown in Figures 3A, 3B and 3C (black traces). Magnetically aligned samples produce well-aligned EPR spectra with the observed orientation-dependent hyperfine splittings with narrower line widths. The lineshape and orientation dependent hyperfine splittings are consistent with the previously published EPR data on the M28 peptide and 5-doxylstearic acid (5-DSA) via magnetically alignment study.^{12, 32, 33} Inspection of CW-EPR spectra of randomly dispersed samples revealed that Q13TOAC and A14TOAC have significant lineshape differences having higher amplitude of left peaks when compared to the randomly dispersed samples of the other substitutions. These data further revealed the slight variation in the spectral line broadening among the powder pattern spectra of A7TOAC, V15TOAC, C16TOAC, L17TOAC, and L18TOAC.

The EPR spectra of the parallel aligned bicelles show three clear peaks and have experimental hyperfine splitting values of 26.2 G for I7TOAC, 26.6 G for Q13TOAC, 27 G for A14TOAC, 25.6 G for V15TOAC, 26.1 G for C16TOAC, 26.6 for L17TOAC, and 27.2 G for L18TOAC. These values are consistent with the previously published experimental hyperfine splitting values for the parallel aligned EPR spectra of magnetically and mechanically aligned samples.^{12, 32–34} These data also reveal that several positions have

more limited motion than others. Substitutions at sites 7, 13, 15 all have a similar, slightly higher motion which 14, 16, 17 and 18 have slightly lower motion. However, all labeled positions have very rigid dynamics. Additionally, a slight difference in the hyperfine splitting, ± 1 G, is observed between the calculated values $A_{\parallel}$ and A_{exp} , measured from the EPR spectra of aligned samples; this indicates that the helical long axis of the peptide is not quite collinear to the slight tilt of the peptide away from the bilayer but rather, it is tilted at a small angle away.

In order to further determine the dynamics of these variants, nonlinear least square analysis (NLSL) microscopic order macroscopic disorder (MOMD) simulation program developed by Freed group^{35, 36} was used to simulate all the spectra following the procedure previously reported in the literature.^{12, 32, 33} All the simulated spectra are included as red traces in Figures 2 and 3. The fits were performed by the rotational diffusion rate, the coefficient of ordering potential and the director tilt angle (ψ) as input varying parameters. The principle hyperfine tensors ($A = [5.6 \text{ G}, 5.6 \text{ G}, 33.3 \text{ G}]$) and the g-tensors ($g = [2.0096, 2.0064, 2.0027]$) were obtained from the literature and held constant for all the spectra during the fitting procedure as suggested in the literature.¹² The rotational correlation time was determined from the rotational diffusion tensor using the method previously described in the literature.¹² Table 1 shows the rotational correlation times (τ_c) and order parameters (S) for all the CW EPR spectra determined from the best fit simulations. These parameters are consistent with the previously reported correlation times and order parameters for the AchR M28 peptide using magnetically and mechanically alignment techniques.^{12, 32, 33} Table 1 reveals that the parallel aligned spectra are simulated with larger correlation times indicating slower motion when compared to that of unaligned spectra. The presence of Tm^{3+} in the flipped bicelles for the case of aligned sample may be causing the slower dynamics.¹² The rotational correlation times vary between 11 ns to 27 ns for the EPR spectra of aligned samples having I7 with least value (11 ns) and L18 with highest value (27 ns), and from 9 ns to 12 ns for the EPR spectra of randomly dispersed samples. The correlation times suggest that the TOAC spin label is rigidly bound at the L18 position, while TOAC is more disordered at the I7 position. This result is consistent with the literature and the structure of the AchR M28 peptide that the C-terminus is more rigid when compared to the N-terminus segment.¹² The order parameters (S) vary between 0.4 to 0.7 for the EPR spectra of aligned samples and 0.2 to 0.5 for the EPR spectra of randomly dispersed samples. The order parameters are also consistent with the rotational correlation times indicating that the N-terminus are disordered while the C-terminus is more ordered. The rotational correlation times and order parameters values also reveal the slight variation in the dynamics of different construct depending upon the TOAC labeled sites.

Determination of the helical tilt angle

Determining the helical tilt angle is very important to understand the structural topology of a protein/peptide with respect to the membrane. In this study, we investigated seven different sites on the M28 peptide for determining the helical tilt angle of the peptide. Using the system of equations and procedure presented in Inbaraj et al., the helical tilt angle for each of the peptides was calculated from corresponding EPR spectra as shown in Table 2.^{12, 14} Simulations of the spectra were also accomplished using the NLSL simulation program

developed by Freed group.^{35, 36} The average helical tilt value obtained from the simulations were consistent with the experimentally determined average helical tilt. Table 2 shows that the helical tilts calculated from each spectrum are varying between $9^{\circ} \pm 4^{\circ}$ to $17^{\circ} \pm 4^{\circ}$ and are consistent within the uncertainties. The average helical tilt is obtained as $\sim 13^{\circ}$. This value is in good agreement with the previously reported values of 12° determined from solid state NMR study, 14° determined from molecular dynamics studies and EPR study via mechanically and magnetically aligned techniques.^{12, 37, 38} These helical tilt data also suggest that the AchR M26 peptide is interacting with lipid bilayers as a transmembrane helix and is nearly parallel to the membrane bilayer normal.

4. Discussions

Rigid spin-labels such as TOAC provide an excellent means to assess the peptide backbone motion and also simplify the dynamics of peptides using alignment methodologies. This dynamic information is otherwise lost in the CW-EPR lineshape of randomly dispersed samples. By rigid labeling and aligning many substitutions, a full assessment can be made about the backbone dynamics and the precise helical tilt. By comparing the EPR spectra of the randomly dispersed and the parallel aligned TOAC labeled peptide samples (Figures 2 and 3), a clear orientation dependence was evident for all spectra. It is obvious that investigating CW-EPR spectra in this fashion essentially allows the deconvolution and enhancement of the data obtained from complicated dynamics and topology of a protein using EPR spectroscopy. From the results of the CW-EPR lineshapes of randomly dispersed samples the TOAC Q13 was able to be distinguished from the other substitutions. Observing the parallel aligned spectra conceivably indicate a clearer distinction between rigid and slightly rigid cases. The lineshape corresponding to the mutants are clearly distinguished from each other with correlation times varying between 11 ns to 27 ns that depend upon the rigidity. The variation in the longer correlation times suggests that the rigidity of the sites is not at the same level, but varies which may be due to the backbone fluctuations of the peptide. In this case, only one component of the dynamics is present and thus simplifies the analysis considerably. Most interestingly, the differences between the substitutions in the parallel-aligned samples are far more evident than in their randomly dispersed counterparts (see Figures 2, 3, and Table 1). The linewidth differences in the randomly dispersed substitutions yielded minimal differences advocating the usefulness of aligned spectra for differentiating subtle differences between amino acid substitutions. The rotational correlation times and order parameters reported for different AchR M26 substitutions indicate that the TOAC labeled sites have varying motions having C-terminus more ordered about the site L18 when compared to the C-terminus about the site I7. Our recent electron spin echo envelope modulation (ESEEM) EPR studies suggested that the AchR M26 peptide may have a helical kink around the Leu10 position in lipid bilayers. It is also noted that the contributions from the rotational correlation time and order parameter are always challenging to get unambiguously resolved using the simulation of EPR spectra at single frequency. So more accurate dynamic parameters may be obtained by performing these experiments at multiple frequency and analyzing the EPR spectral data simultaneously.

Besides the dynamic and backbone assessment, labeling and aligning a large number of AchR M26 substitutions allowed us to precisely map the helical tilt. Previously the degree of

variance from one amino acid to the next was not known. Here we experimentally establish that the helical tilt does not vary heavily if the peptide is roughly parallel to the membrane normal. The variance in helical tilt is most likely higher for peptides that lay on the surface of the membrane. In the ^{15}N solid state nuclear magnetic resonance (SSNMR) alignment studies, only one ^{15}N labeled amide yields an estimate of helical tilt and more data points are needed for the accurate measurement.¹² Here we determined the more accurate helical tilt angle by utilizing several data points in a similar fashion. The helical tilt reported in this study is also consistent with the previously reported helical tilt $\sim 13^\circ$ of a similar second transmembrane (TM2) domain of GABAA receptor studied using solid-state NMR and MD simulations.³⁹ The site specific helical tilts reported in this study can be further used as orientation dependent EPR restraints that could be implemented in the simulated annealing structure calculations as well as in the restrained MD simulations to gain deeper insights about structure and dynamics to move the EPR approach beyond the derivation of single helical tilt. The EPR magnetically alignment method is well suited for answering the pertinent question about the deviation in the helical tilt due to hydrophobic mismatch of different lipid bilayer thickness similar to that of SSNMR technique.^{12, 40, 41} While this magnetically membrane alignment method has been explored only for the small peptide in this paper, it can be potentially applied to any membrane protein of unlimited size by using the more rigid bifunctional spin-label (BSL) and bacterial over expression.^{42–46}

5. Conclusions

We successfully determined accurate helical tilt angle and dynamics of the AchR M26 peptide by utilizing the magnetically aligned lipid bilayers (bicelles) EPR technique and multiple TOAC labeled peptide substitutions. By using this alignment method we measured an average helical tilt of $13^\circ \pm 4^\circ$ for AchR M26 peptide in lipid bilayers. Our data also indicated the more resolved local backbone dynamics information for the TOAC labeled sites in the aligned samples when compared to the randomly dispersed samples. Alignment methodologies provide a useful tool to extract and simplify valuable information from a dynamically rich CW-EPR spectra. The results presented in this paper illustrate the breadth of applications that can be utilized from aligned data. While this study only encompasses a small peptide, it is expandable to larger proteins and more complicated systems.

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Highlights

1. Accurate helical tilt angle was determined using magnetically aligned lipid bilayers (bicelles) coupled with EPR spectroscopy and multiple TOAC labeled peptide substitutions.
2. More resolved local backbone dynamics information for the TOAC labeled sites was obtained in the aligned samples when compared to randomly dispersed samples.
3. Magnetically aligned lipid bilayers can be very useful for studying structural and dynamic properties of membrane proteins using EPR spectroscopic techniques.

Figure 1.

(A) Chemical structure of the TOAC spin label probe, (B) Schematic representation of the AchR M26 peptide with the TOAC spin labeling sites represented by green spheres at their alpha carbons in a transmembrane orientation in the phospholipid bilayers. n represents the phospholipid bilayer normal, and B_0 represents an applied magnetic field.

Figure 2.

CW-EPR spectra of TOAC labeled M28 residues I7, Q13, A14, and V15. The top spectra are standard (randomly dispersed) CW-EPR spectra in DMPC vesicles. The bottom spectra are DMPC:DHPC bicelles aligned such that the bilayer is parallel with respect to the magnetic field ($n\parallel B_0$). The black traces are experimental EPR spectra and red traces are theoretically simulated EPR spectra.

Figure 3. CW-EPR spectra of TOAC labeled M28 residues C16, L17, and L18. The top spectra are standard (randomly dispersed) CW-EPR spectra in DMPC vesicles. The bottom spectra are DMPC:DHPC bicelles aligned such that the bilayer is parallel with respect to the magnetic field ($n||B_0$). The black traces are experimental EPR spectra and red traces are theoretically simulated EPR spectra.

Table 1

Rotational correlation time and order parameters (S) for parallel aligned (||) and randomly dispersed samples obtained from NLSL MOMD spectral simulation of EPR spectra. The uncertainties introduced in τ_c values are ± 2 –3 ns and S values are ± 0.1 –0.2.

sites		Correlation time (τ_c , ns)	S
7	aligned	11	0.4
	random	9	0.4
13	aligned	12	0.5
	random	13	0.4
14	aligned	19	0.7
	random	10	0.2
15	aligned	12	0.6
	random	9	0.4
16	aligned	15	0.4
	random	10	0.4
17	aligned	23	0.7
	random	11	0.5
18	aligned	27	0.6
	random	12	0.4

Table 2

Helical tilt angles calculated from the EPR spectra from Figure 2 and 3. The uncertainties in helical tilt angles are $\pm 4^\circ$.

M26 Substitution	Helical Tilt
I7	11°
Q13	17°
A14	9°
V15	17°
C16	16°
L17	11°
L18	9°