

# Fourier Transform Coupled to Tryptophan-Scanning Mutagenesis: Lessons from its Application to the Prediction of Secondary Structure in the Acetylcholine Receptor Lipid-Exposed Transmembrane Domains

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**ABSTRACT** Although Fourier transform (FT) and tryptophan-scanning mutagenesis (TrpScanM) have been extremely useful for predicting secondary structures of integral membrane proteins, they are deemed to be low-resolution techniques. Herein, we describe the combined use of FT and TrpScanM (FT-TrpScanM) as a more reliable approach to the prediction of secondary structure. Five TrpScanM studies of the acetylcholine receptor lipid-exposed transmembrane domains (LETMDs) were revisited. FT analysis of the raw data from the aforementioned TrpScanM studies supports and validates the conclusions derived from the tryptophan-periodicity profiles, and suggests that similar findings could be obtained from fewer tryptophan substitutions than in previous studies. Furthermore, by FT-TrpScanM, we were able to determine the minimum number of consecutive tryptophan substitutions necessary for more robust prediction of  $\alpha$ -helical secondary structures. We also examined the quality of structure predictions for LETMDs. Finally, this study encourages future utilization of FT-TrpScanM to more reliably predict secondary structures of the membrane protein LETMDs.

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Unlike globular proteins, high-resolution three-dimensional structures of membrane proteins as determined by x-ray crystallography are seldom found in protein structure databases, reflecting the difficulties associated with their crystallization. As preferred alternatives to x-ray crystallography for structural assessment, NMR and cryo-electron microscopy have been typically used; however, they are limited by protein size and reachable resolution, respectively. In this scenario, the tryptophan-scanning mutagenesis (TrpScanM) has emerged as another option for predicting the secondary structure of lipid-exposed transmembrane domains (LETMDs), which can then be used to model their overall spatial orientation (1). Although TrpScanM has been successfully applied to several ion-channel proteins (e.g., J.D. Otero-Cruz *et al.*, 2007), TrpScanM is, nevertheless, deemed a low-resolution method for predicting secondary structure because it does not provide direct structural information. Herein, we describe a detailed study on the prediction of secondary structure of the acetylcholine receptor (AChR) LETMDs coupling Fourier transform (FT) to TrpScanM (FT-TrpScanM). With this approach, we were able to provide new structural information, estimate mean periodicities, support and validate the results from TrpScanM, determine the minimum number of consecutive tryptophan substitutions required to predict  $\alpha$ -helical structures, assess the quality of structure predictions, and present FT-

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TrpScanM as a valuable alternate tool to reliably predict secondary structures of membrane protein LETMDs.

The application of TrpScanM to AChR consisted of systematic substitution of a series of residues by tryptophan along the LETMDs and characterizing the functional consequences by two-electrode voltage clamp using several acetylcholine concentrations to determine ACh EC<sub>50</sub> values for each dose-response curve. By plotting the ACh EC<sub>50</sub> values as a function of the tryptophan substitution positions and using a cubic spline curve to interpolate the points, tryptophan-periodicity profiles (TrpPPs) were generated and used to estimate the periodicities of the oscillation patterns (Fig. 1 *A-E*). The rationale for choosing TrpScanM is that tryptophan substitutions along the LETMDs should result in a loss of function when facing the protein interior, whereas when facing the lipid they should be innocuous, giving rise to periodic patterns of perturbations in ion-channel function.

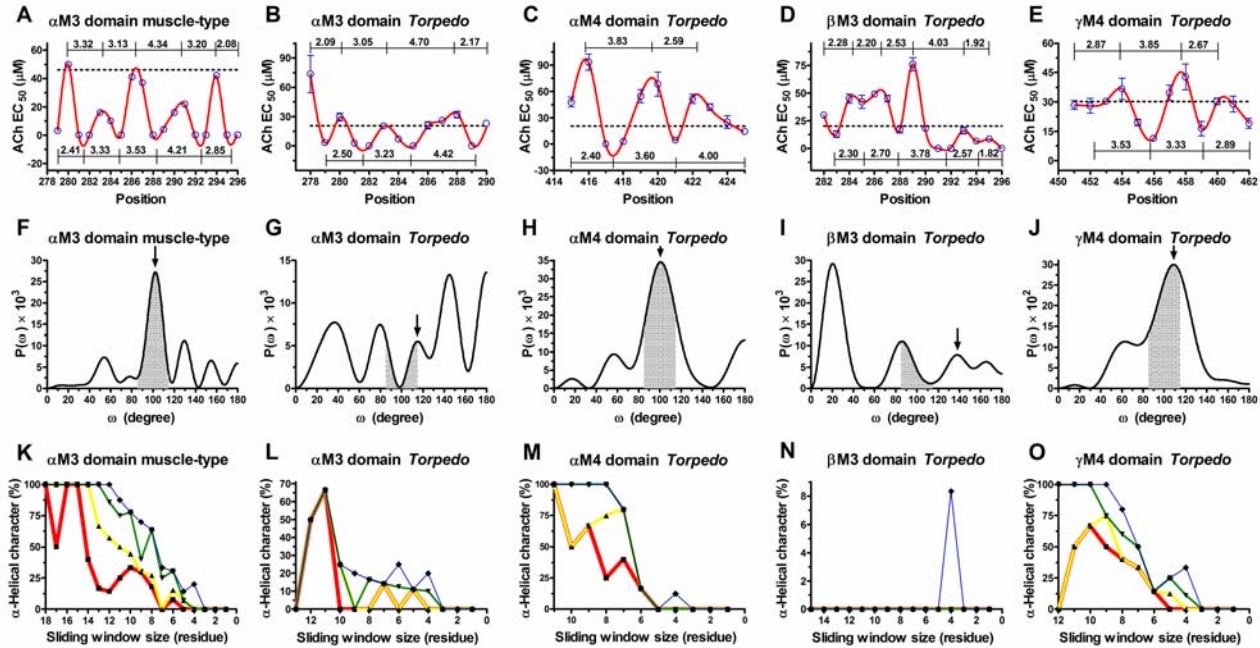
In this letter, we revisited the reported TrpScanM from five LETMDs ( $\alpha$ M4,  $\beta$ M3,  $\gamma$ M4, and two  $\alpha$ M3 domains) of two different AChR species. The raw data of these studies, which predicted helical structures with diverse periodicities and packing arrangements for the LETMDs (2-6), were used to generate the FT power spectra and the  $\alpha$ -helical character curves. The sequences of ACh EC<sub>50</sub> values were evaluated by a least-squares discrete FT equation to produce the FT power spectra and estimate the mean

periodicities (Fig. 1 *F-J*). The FT power spectra  $P(\omega)$  were plotted as a function of angular frequency  $\omega$  (rotation angle between residues around a helical axis) using

$$P(\omega) = \left[ \sum_{j=1}^N (V_j - \langle V_j \rangle) \cos(j\omega) \right]^2 + \left[ \sum_{j=1}^N (V_j - \langle V_j \rangle) \sin(j\omega) \right]^2$$

where  $V_j$  is the ACh EC<sub>50</sub> value at a given position  $j$ ,  $\langle V_j \rangle$  is the mean value of  $V_j$  in the sliding window, and  $N$  is the

number of ACh EC<sub>50</sub> values in the sliding window (7). The sliding windows must comprise consecutive ACh EC<sub>50</sub> values. For instance, the FT analysis of a sequence of values from an ideal  $\alpha$ -helical pattern (3.6 residues per turn) should give a prominent peak at  $\sim 100^\circ$  in the FT power spectrum, given that the  $\omega$  parameter is related to the number of residues per turn ( $d$ ) by  $\omega = 360^\circ/d$ .



**FIGURE 1.** Tryptophan-periodicity profiles, Fourier transform power spectra, and  $\alpha$ -helical character curves generated from tryptophan-scanning mutagenesis data of the AChR lipid-exposed transmembrane domains. (*A-E*) are TrpPPs. Values *along the lines* indicate the number of residues per helical turn between adjacent maximums and minimums peaks. The *black dashed line* indicates the ACh EC<sub>50</sub> value of the wild-type AChR. (*F-J*) are FT power spectra from entire sequences of the ACh EC<sub>50</sub> values shown in the TrpPPs (*A-E*). The *black headed arrows* indicate the peaks that correspond to mean periodicities (*A-E*). *Grey shadings* demarcate the region  $85^\circ \leq \omega \leq 115^\circ$  used to calculate peak ratios. (*K-O*) are  $\alpha$ -helical character curves of different periodicity intervals as a function of the number of residues in the sliding window; *red line* (3.5 – 3.7) residues/turn; *yellow line* (3.4 – 3.8) residues/turn; *green line* (3.3 – 3.9) residues/turn; or *blue line* (3.2 – 4.0) residues/turn.

Using FT, the sequences of ACh EC<sub>50</sub> values were decomposed into multiple peaks with different abundances at the FT power spectra, suggesting that the TrpPPs may be divided as well into several periodicities with diverse oscillation patterns. The mean periodicities of the sequences of ACh EC<sub>50</sub> values were estimated from the peak localized in the vicinity of the rotation angle indicated by the mean periodicities of the TrpPPs (see *black headed arrow* in Fig. 1 *F-J* and Table 1). The similarity between the mean periodicities determined by both methods suggests that FT supports and validates the TrpScanM. Interestingly, the prominent peaks of the *Torpedo*  $\alpha$ M3 and  $\beta$ M3 AChRs were not found in the expected vicinity of the rotation angle indicated by the mean periodicities of the TrpPPs. The unexpected location of these prominent peaks could be due to the contribution of aberrant oscillations in the TrpPPs.

To investigate the reliability of the ACh EC<sub>50</sub> values for predicting  $\alpha$ -helical periodicities, we calculated the ratio of  $P(\omega)$  in the  $\alpha$ -helical range ( $85^\circ \leq \omega \leq 115^\circ$ ) relative to  $P(\omega)$  over the whole spectrum using

$$\text{Peak ratio} = \left[ \int_{85^\circ}^{115^\circ} P(\omega) d\omega \right] / \left[ \int_0^{180^\circ} P(\omega) d\omega \right]$$

where  $P(\omega)$  is the FT power spectrum. *Peak ratio* values greater than 2, which represent at least 33% of the whole power spectrum, have been considered a very good indicator of an  $\alpha$ -helical secondary structure (7). However, the *peak ratio* parameter is not enough for a clear prediction because the prominent peak maximum could be located outside the  $\alpha$ -helical frequency interval ( $85^\circ \leq \omega \leq 115^\circ$ ) and yet have a *peak ratio*  $> 2$ . In order to avoid this problem and to more reliably predict secondary structures, we generated  $\alpha$ -helical character curves (8).

**TABLE 1. Secondary structure parameters of AChR lipid-exposed transmembrane domains determined by tryptophan-periodicity profiles and Fourier transform power spectra.\***

AChR type	№ residues	Tryptophan-periodicity profiles		Fourier transform power spectra		
		Periodicity <i>residues/turn</i>	Expected rotation angle <i>degree</i>	Rotation angle <i>degree</i>	Peak ratio	Expected mean periodicity <i>residues/turn</i>
$\alpha$ M3 Muscle-type	18	3.24 $\pm$ 0.70	111.11	102.25	2.94	3.52
$\alpha$ M3 <i>Torpedo</i>	12	3.17 $\pm$ 1.04	113.56	115.00	0.53	3.13
$\alpha$ M4 <i>Torpedo</i>	11	3.28 $\pm$ 0.74	109.76	100.76	3.15	3.57
$\beta$ M3 <i>Torpedo</i>	15	2.61 $\pm$ 0.74	137.93	138.06	0.75	2.61
$\gamma$ M4 <i>Torpedo</i>	12	3.19 $\pm$ 0.45	112.85	108.88	2.67	3.31

\* Given values correspond to analysis performed on the entire sequence of ACh EC<sub>50</sub> values from the AChR LETMDs. Periodicities of the TrpPPs are given as mean  $\pm$  SD. Expected rotation angles of the TrpPPs and the expected mean periodicities of the peaks that correspond to mean periodicities of the TrpPPs were calculated using  $periodicity$  (residues/turn) =  $360^\circ/rotation\ angle$ .

To generate  $\alpha$ -helical character curves, the sequences of ACh EC<sub>50</sub> values were partitioned to form sliding windows of different sizes (number of sliding windows =  $n(n+1)/2$  “triangular number”, where  $n$  is the number of values in the sequence; e.g., four values produce ten different sliding windows). FT power spectra for different sliding windows were generated to determine the percent of FT power spectra per sliding window size that fulfilled the two parameters: (i) peak ratio > 2 and (ii) prominent peak inside several frequency intervals such as  $102.9^\circ \leq$  prominent peak  $\leq 97.3^\circ$  for 3.5-3.7 periodicities;  $105.9^\circ \leq$  prominent peak  $\leq 94.7^\circ$  for 3.4-3.8 periodicities;  $109.1^\circ \leq$  prominent peak  $\leq 92.3^\circ$  for 3.3-3.9 periodicities; and  $112.5^\circ \leq$  prominent peak  $\leq 90^\circ$  for 3.2-4.0 periodicities (Fig. 1 K-O). The  $\alpha$ -helical character curves of the muscle-type  $\alpha$ M3 domain, and the *Torpedo*  $\alpha$ M4 and  $\gamma$ M4 domains were more reliable in predicting  $\alpha$ -helix structures than those of the *Torpedo*  $\alpha$ M3 and  $\beta$ M3 domains because they maintain 100%  $\alpha$ -helical characters even while reducing the size of the sliding windows. The *Torpedo*  $\alpha$ M3 and  $\beta$ M3 domains displayed low percents in the  $\alpha$ -helical character curves, presumably due to two factors: (i) insufficient amount of tryptophan substitutions to predict an  $\alpha$ -helix structure and/or (ii) the sequence of values predicted a mixture of  $\alpha$ - and  $3_{10}$ -helix structures or a  $3_{10}$ -helix structure. The size of the smallest sliding window displaying a level of 100%  $\alpha$ -helical character can be considered indicative of the minimum number of consecutive tryptophan substitutions necessary to predict reliable  $\alpha$ -helix structures. In essence, the quality of structure predictions could be examined by the  $\alpha$ -helical character curves of the different periodicity intervals.

In conclusion, FT-TrpScanM may be a useful tool for predicting the secondary structure of membrane protein LETMDs, shedding light into their overall spatial orientation. The  $\alpha$ -helical character curves may be used to fingerprint the quality of predictions and determine the minimum of substitutions required for reliable predictions. Finally, this work corroborates the predictions from

previous TrpScanM studies and suggests that similar results could have been reached with even fewer substitutions, encouraging future utilization of FT-TrpScanM as means to predict secondary structures of LETMDs.

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## REFERENCES AND FOOTNOTES

1. The abbreviations used are: AChR, acetylcholine (ACh) receptor; FT, Fourier transform; FT-TrpScanM, Fourier transform coupled to tryptophan-scanning mutagenesis; LETMDs, lipid-exposed transmembrane domains; TrpPPs, tryptophan-periodicity profiles; TrpScanM, tryptophan-scanning mutagenesis.
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