# Investigating the Secondary Structure of Membrane Peptides Utilizing Multiple ${ }^{2} \mathrm{H}$-labeled Hydrophobic Amino Acids via Electron Spin Echo Envelope Modulation (ESEEM) Spectroscopy 

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## Supplemental Figures:



Figure S1: Three-pulse ESEEM experimental data of AChR M2 $\mathbf{~} \delta$ with ${ }^{2} \mathrm{H}$-labeled $\mathrm{d}_{3}$ Ala6 at the N-terminal (-) and C-terminal (+) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{T}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.

Ala 12


Figure S2: Three-pulse ESEEM experimental data of AChR M2ס with ${ }^{2}$ - -labeled $\mathrm{d}_{3}$ Ala12 at the N-terminal (-) and C-terminal (+) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{T}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.


Figure S3: Three-pulse ESEEM experimental data of AChR M2 2 with ${ }^{2} \mathrm{H}$-labeled $\mathrm{d}_{3}$ Ala14 at the N-terminal (-) and C-terminal (+) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{T}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.


Figure S4: Three-pulse ESEEM experimental data of AChR M2 with $^{2}{ }^{2}$-labeled $\mathrm{d}_{8}$ Val9 at the N -terminal (-) and C-terminal (+) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{r}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.


Figure S5: Three-pulse ESEEM experimental data of AChR M2 $\delta$ with ${ }^{2} \mathrm{H}$-labeled $\mathrm{d}_{8}$ Val15 at the N-terminal (-) and C-terminal (+) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{T}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.


Figure S6: Three-pulse ESEEM experimental data of AChR M2 2 with ${ }^{2} \mathrm{H}$-labeled $\mathrm{d}_{8}$ Phe16 at the N-terminal ( - ) and C-terminal ( + ) sides in DMPC/DHPC (3.5:1) bicelles at $\mathrm{T}=200 \mathrm{~ns}$ for the $i+1$ to $i+4$ in Time domain and Frequency domain.


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