# $^{19}$F Paramagnetic Relaxation-Based NMR for Quaternary Structural Restraints of Ion Channels

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**Supporting Information**

**Table 1S.** $^{19}$F PRE NMR measured inter-subunit distances at the selected residues in α7nAChR are comparable with distances at equivalent positions in the cryo-EM structure of the resting-state 5-$HT_{3A}$ receptor (PDB code: 6BE1), considering that small distance discrepancies are expected due to the different reference points. As observed with L253C in ELIC, compared to the distance measured between Cβ atoms of two adjacent equivalent residues in crystal structures, an additional 2-3 Å is expected in the distances measured by $^{19}$F PRE NMR or DEER ESR due to extra lengths of labeled probes.

| **Residues of α7nAChR** | **$^{19}$F PRE NMR measured distances (Å)** | **Equivalent residues of 5-$HT_{3A}$ receptor** | **Cβ-Cβ distances (Å) in the cryo-EM structure** |
|---|---|---|---|
| C435 | 18.3 ± 1.7 | A423 | 16.0 ± 0.1 |
| C427 | 17.2 ± 1.6 | K415 | 15.5 ± 0.2 |

**Determination of rotational correlation time ($\tau_c$)**

To determine a rotational correlation time ($\tau_c$) for the α7nAChR TMD+ICD, we collected a series of 1D [$^{15}$N-$^{1}$H]-TRACT NMR spectra[21] with varied relaxation periods of 0.1, 0.5, 1, 2, 4, 8, 16, 32, and 64 ms. The experiments were performed with a recycle time of 1 s at 45 ºC on a Bruker Avance 700 MHz spectrometer equipped with a triple-resonance inverse-detection cryoprobe TCI (Bruker Instruments). The relaxation rates for the α- and β-spin states ($R_\alpha$ = 43 ± 16 Hz and $R_\beta$ = 200 ± 62 Hz) were obtained by fitting an exponential intensity decay for peaks in 8.55 – 8.95 ppm (helical region) of the TRACT spectra in the α and β states[21] as a function of the relaxation time. The TRACT NMR data collected at 45 ºC for the α7nAChR TMD+ICD resulted in $\tau_c$ = 65.6 ns, which matches reasonably well with the result ($\tau_c$ = 69.1 ns at 45 ºC) obtained independently from an empirical calculation based on the Stokes' law{Cavanagh, 1996 #255} (http://nickanthis.com/tools/tau.html; Cavanagh, Fairbrother, Palmer, Rance, Skelton. Protein NMR Spectroscopy: Principles and Practice, 1996, Academic Press, San Diego) and the molecular weight of the protein in micelles (183.5 kDa). Using the same calculation tool and the experimental value measured at 45 °C, we obtained $\tau_c$ = 161.6 ns at 10 °C, which was the temperature used for $^{19}$F PRE NMR. A higher temperature, such as 45 ºC, shortens the lifetime of the labeled probes and thus is prohibited in $^{19}$F PRE NMR. A low temperature, such as 10 °C, is inadequate for TRACT NMR because of a poor signal/noise ratio and poor spectral resolution. For ELIC, $\tau_c$ = 218 ns at 10 °C was obtained from the empirical calculation based on the Stokes' law{Cavanagh, 1996 #255} and the estimated protein–micelle molecular weight (235 kDa). Its larger molecular weight impairs the quality of TRACT NMR spectra for extracting useful $\tau_c$ information at either 10 ºC or 45 ºC.