**19F-RAI-CODEX Investigation on Gramicidin A**

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The CODEX (center band only detection of exchange) NMR experiment is suitable for characterizing slow dynamics of protein side chains. Here we present the incorporation of the RAI-principle (recoupling of anisotropy information) into the CODEX experiment, as shown in Figure 1.

**Fig. 1:** RAI-CODEX sequence: DD...dipolar decoupling, TPPM...two pulse phase modulation, \(\pi/2\)...90° pulse , \(\pi\)...180° pulse, \(\tau_m\)...mixing time, \(\tau_z\)...z-filter time, \((2n-1)t_r\)...odd number of rotor periods.

The sequence starts with a 90° excitation pulse, followed by a four-pulse RAI-sequence for recoupling the chemical shift tensor during an odd number of rotor periods. The magnetization is then stored onto the z-direction for a period of \(\tau_m\), during which the \(^{19}\text{F}\) tensor orientation may change due to slow motion. After the magnetization is brought back into the x-y-plane and the chemical shift anisotropy is recoupled again by the four-pulse RAI-sequence, followed by a z-filter with a short time of \(\tau_z\). For each exchange time \(\tau_m\) two experiments (with center band intensities \(S_i\) and \(S_0\)) are taken by changing the order of \(\tau_m\) and \(\tau_z\). The time evolution of the ratio of the intensities \(S_i\) and \(S_0\) gives rise to dynamics information. For jumps of mobile side chains the number of magnetically in-equivalent orientations can be obtained. The equilibrium ratio \((S_i/S_0)\) is the inverse of the number of detectable states.

The motion of Gramicidin A (gA) in membranes, its interaction with the lipids and the conformation as well as the mobility of the tryptophan side chains have been intensively studied in the literature. Here, we applied the RAI-CODEX sequence to 5F-Trp\(_{13}\)-gA in lipid bilayers to study the motion of the Trp side chain. The \(^{19}\text{F}\)-RAI-CODEX curve of 5F-Trp\(_{13}\)-gA is given in Figure 2. The equilibrium value is 0.25, an indication of 4 different orientations for the Trp\(_{13}\) side chain. The decay time is around 40ms which tells us that the hopping between these four sites is rather slow.

**Fig. 2:** \(^{19}\text{F}\)-CODEX of 5F-Trp\(_{13}\)-gA curve with a single decay time of \((41\pm6)\) ms and an equilibrium value of \(0.25\pm0.03\).

**References**