^{19}F/^{1}H Solid State NMR Probe for Small Volumes of Mechanically Aligned Proteins

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Introduction

Solid state ^{19}F NMR is as a powerful tool for investigating the structure and dynamics of proteins and other biological systems [1]. However, the Larmor frequencies of both nuclei in the ^{19}F–^{1}H experiments are high enough to induce substantial heating inside biological samples. This radio frequency (RF) heating can severely undermine integrity of the sample during the acquisition [2]. Here we report a ^{19}F–^{1}H solid state NMR probe, a collaborative effort between the Forschungszentrum Karlsruhe (FZK) and the National High Magnetic Field Laboratory. This 600 MHz probe was designed to overcome destructive sample heating at both frequencies. Dimensions of the coil and sample package were optimized for those mechanically aligned protein preparations where amount of the sample is limited by high cost of protein expression.

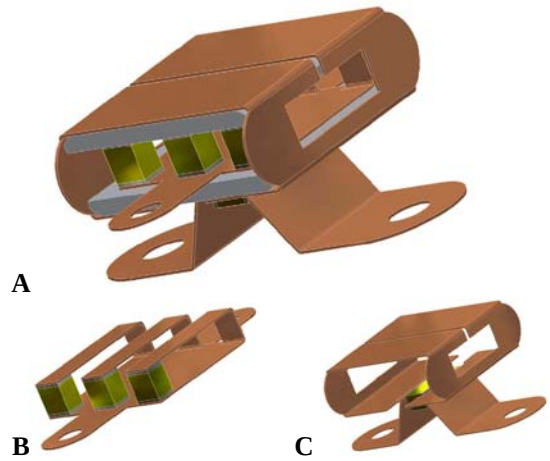

Fig. 1. (A) Nested ^{19}F–^{1}H low-E RF assembly for the limited sample volume: 7×9×3 mm. (B) Three-fold ^{19}F single-gap resonator, which is inserted inside a ^{1}H loop-gap resonator [5] (C).

Methods

Construction of the instrument was performed at NHMFL and finalized at FZK, where the probe will reside in the group of Prof. Ulrich. The probe was based on the recently introduced low-E design [3, 4], which utilizes 2 orthogonal loop-gap resonators in order to reduce the conservative electric fields that cause heating in conductive samples. These resonators are nested, with the ^{19}F resonator placed on the inside to provide the greatest sensitivity (Fig. 1). The radio frequency field homogeneity over the 7×9×3 mm sample volume was measured as the ratio of 810° to 90° pulse amplitudes for both ^{19}F and ^{1}H channels. The ratios were found to be 87% in the ^{19}F and 96% in the ^{1}H channels, indicating highly homogeneous RF fields that benefit the cross-polarization experiments. RF fields $\omega_1/2\pi$ = 100 kHz were achieved at 96 W of input power in the ^{19}F channel and at 592 W in the ^{1}H channel. Isolation between the ^{19}F and ^{1}H ports provided within the probe was measured as 26 dB at the ^{1}H frequency and 34 dB at the ^{19}F frequency. Further isolation of at least 90 dB was achieved by additional external filters.

Results

Figure 2 shows an experimentally obtained two-dimensional ^{19}F CPMG spectrum of an anti-inflammatory drug – Flufenamic acid (FFA) in DMPC bilayers aligned in aluminium nanopores (1:16 molar ratio, fully hydrated, at 35°C). Four 180º pulses were used with an XY-4 phase cycling scheme [6] to minimize the 180º pulse imperfections, giving rise to an effective dwell time of 18.75 µs in the t_1 dimension. An echo time of 10 µs was used for each t_1 increment. RF field magnitudes ($\omega_1/2\pi$) of 100 and 70 kHz were used for ^{19}F and for ^{1}H decoupling, respectively.

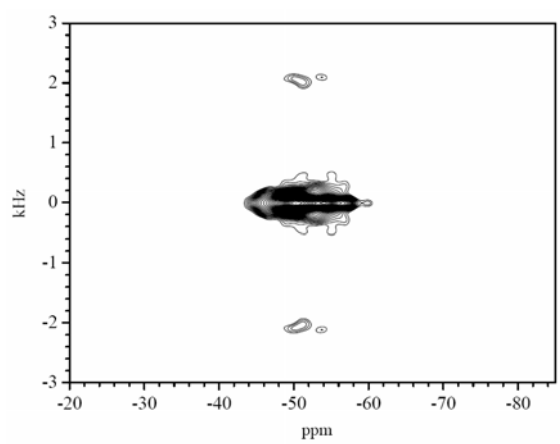

Fig. 2. 2D ^{19}F CPMG spectrum of Flufenamic acid in DMPC (1:16) in aluminum nano-pores. RF field strengths: ^{19}F B_1 = 100 kHz, ^{1}H B_1 for decoupling = 70 kHz.

Acknowledgements

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References

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