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# Application of the $^{19}$F-Waterlogsy Type Experiment for NMR-Based Screening of Fluorinated Compounds

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## Abstract

The WaterLOGSY type experiment with $^{19}$F detection was applied to observe the interaction between a fluorinated compound and a macromolecule. The proposed experiment, which was developed based on the $^{19}$F{$^{1}$H} saturation transfer difference experiment, was carried out using the conventional spectrometer equipped with a single high band amplifier and a H/F/C-double tuned probe. The selective $^{19}$F detection is advantageous in screening the fluorinated compounds, considering that $^{19}$F is a sensitive nucleus in NMR spectroscopy. The effective approach to discriminate binding of the fluorinated compounds to proteins with $^{19}$F detection was demonstrated using the complex of diflunisal and human serum albumin.

**Keywords:** $^{19}$F NMR; NMR-based screening; Fluorinated compound; WaterLOGSY

## Introduction

NMR spectroscopy has been utilized as a useful method for analyzing macromolecular complexes and screening compounds with affinity to target macromolecules. Various NMR-based screening methods have been developed using chemical shift perturbation [1-3], transferred NOE [4,5] and diffusion and relaxation editing methods [6,7]. In the development of NMR techniques for screening compounds, one of the essential requirements is the selective detection of the ligand signals while suppressing signals from target proteins. It has been shown that NOE-pumping [8], reverse NOE-pumping [9], saturation transfer difference (STD) [10] and water-ligand observed via gradient spectroscopy (WaterLOGSY) [11,12] experiments could directly detect bound ligands. These methods were designed to detect ligands with proton detection, and furthermore, the NMR-based methods have been extended to fluorine detection [13,14]. An incorporation of fluorine into drugs provides simultaneous modulation of electronic, lipophilic and steric parameters, indicating that inclusion of fluorine atoms in a drug molecule can alter its chemical properties and biological activities, and also influence the interaction with its target [15]. In analysis of fluorinated compounds, some useful NMR methods, which are applicable to the NMR-based screening, are required to be developed. Although $^{19}$F NMR spectroscopy is feasible to analyze the fluorinated compounds [16-19], the pervasive NMR spectrometer consoles consist of a single high and a few low band amplifiers, which are incapable of performing $^{1}$H-$^{19}$F heteronuclear experiments. In the recent study, an efficient technique to achieve $^{1}$H-$^{19}$F heteronuclear experiments using a conventional NMR spectrometer equipped with a $^{1}$H/$^{19}$F /$^{13}$C-double tuned probe was developed in our study [20]. Here, we propose an effective approach to discriminate binding of the fluorinated compounds to proteins with $^{19}$F detection, using the complex of diflunisal (Figure 1a) and human serum albumin (HSA).

## Materials and Methods

### Instrumentation and chemicals

All of the spectra were recorded at 20°C on a Varian 600 M*Hz* NMR system or JEOL ECZ-400S spectrometer. Diflunisal, enoxacin (Figure 1b) and HSA were purchased from Sigma-Aldrich. Two NMR samples were prepared; (i) a 5 mm tube containing a solution of 4.0 mM diflunisal and 4.0 mM enoxacin, (ii) double NMR tubes, which comprised a 3 mm tube used as an inner tube containing 10 mM enoxacin and a 5 mm tube used as an outer tube containing 5.0 mM diflunisal and 0.1 mM HSA. The enoxacin solution, which was prepared in the absent of HSA, was used as a negative control. Each solution contained 90% $^{1}H_2O$ and 10% $^{2}H_2O$.

### NMR spectroscopy

The experimental parameters of $^{19}$F{$^{1}$H} STD experiment at $^{1}$H frequency of 600 *MHz* were as follows; data points=8192, spectral width of $^{19}$F=16026 Hz, number of scans=8192, d1=0.1 s, d2=1.5 ms, on-resonance frequencies of $^{1}$H=0.9 or 4.8 ppm, and off-resonance frequency=-20 ppm. In the reference $^{19}$F experiment, number of scans was 64. Those of W5-WaterLOGSY experiment were as follows; data

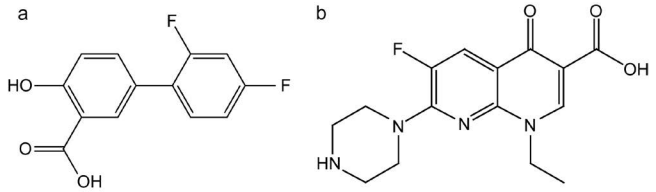


**Figure 1:** Chemical structures of (a) diflunisal and (b) enoxacin.

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points=16384, spectral width of $^{1}$H=8012 *Hz*, number of scans=128, recycle time=1.0 s, mixing time=1.5 s. The Gaussian window function was used with zero-filling by a factor of 2.

## Results and Discussion

### Pulse sequence optimization

Share of one high band amplifier without circuitry changes enabled acquiring the $^{1}$H-$^{19}$F heteronuclear spectra, although the pervasive NMR spectrometer, consisting of a single high and a few low band amplifiers, is incapable of performing $^{1}$H-$^{19}$F experiments. In the present research, the $^{19}$F{$^{1}$H} STD pulse sequence was modified to be applicable to the WaterLOGSY type experiment for screening of fluorinated ligands bound to protein. In comparison with the former type $^{19}$F{$^{1}$H} STD pulse sequence (Figure 2a), the present pulse sequence was rather simplified as shown in Figure 2b. The consecutive $^{19}$F and $^{1}$H 90° pulses between the gradient pulses G1 were omitted, and a single $^{1}$H pulse at the low power was incorporated for the selective excitation of proteins. In the $^{19}$F{$^{1}$H} STD experiment, two type of spectra were acquired depending on the $^{1}$H resonance of selective excitation. In the first experiment, the $^{1}$H resonance at 0.9 ppm, corresponding to the methyl region of protein, was selectively excited. During the subsequent process, the proton magnetization of the protein was transferred to fluorine of the ligand for detection. This experiment corresponds to the conventional STD experiment with $^{19}$F detection, which can also be acquired using a sample solution of 100% $^{2}H_2O$. The $^{19}$F{$^{1}$H} STD spectra acquired using the above pulse sequences are shown in Figure 3. The $^{19}$F chemical shifts of diflunisal were -112.5 and -115.0 ppm, and that of enoxacin was -129.4 ppm. Enoxacin in a 3 mm tube, corresponding to the inner tube, was used as a free ligand in the absence of HSA. Although the $^{1}$H resonance of a methyl group of enoxacin (1.4 ppm) was close to that of the selective excitation (0.9 ppm), an intensity of the $^{19}$F signal of enoxacin resonating at -129.4 ppm in the $^{19}$F{$^{1}$H} STD experiment was minute (Figure 3b). This result indicated that the $^{1}$H selective excitation at 0.9 ppm unexcited $^{19}$F of enoxacin and the $^{19}$F signals of diflunisal bound to HSA were selectively detected. The present optimization of the pulse sequence resulted in the increase of sensitivity by a factor of 1.7.

### $^{19}$F - WaterLOGSY type experiment

In the second experiment, the $^{1}$H resonance at 4.8 ppm, corresponding to the water resonance, was selectively excited. The $^{1}$H magnetization of water, which was transferred to the protein, was again transferred to the bound ligand via direct or indirect relay processes. The signals of enoxacin were expected to be observed with the opposite phase with respect to those of the bound ligand. This experiment is conceptually identical to the WaterLOGSY. In a sample solution without HSA, all $^{19}$F signals of difunial and enoxacin were observed with the same phase as the negative signals (Figure 4a). In the experiments using a sample of double NMR tubes, the $^{19}$F signals of diflunial which bound to HSA and that of enoxacin as a negative control were observed as opposite phase (Figure 4b). The present methods are feasible to distinguish the bound molecules from the unbound molecules. In comparison of the conventional $^{19}$F spectra in the presence and absence of HSA, (Figure 4c and 4d), the signals of diflunial, resonating at -112.8 and -115.0 ppm, were clearly broadened in the presence of HSA (Figure 4c), indicating the complex formation.

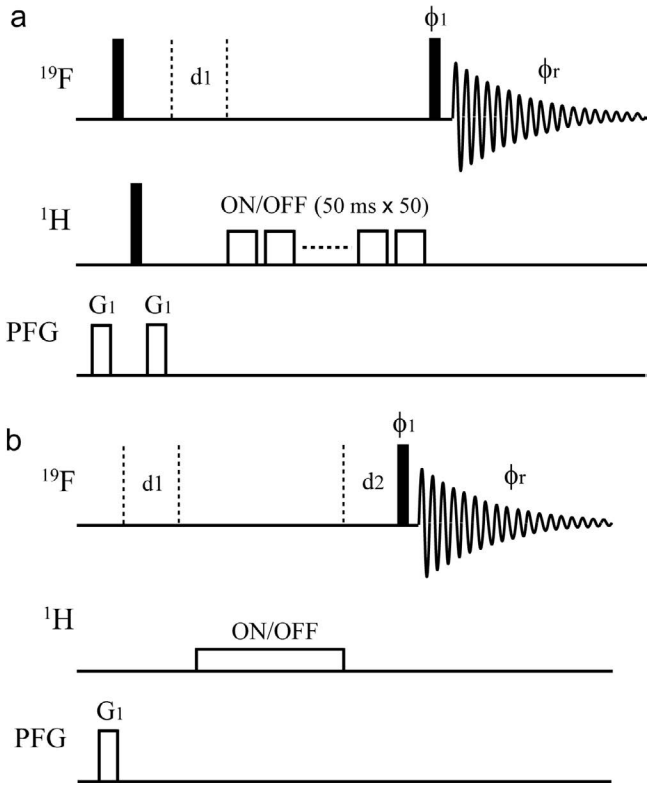


**Figure 2:** Pulse sequences of $^{19}$F{$^{1}$H} STD experiment. (a) The former type pulse sequence and (b) the present $^{19}$F{$^{1}$H} STD pulse sequence. The thin bars represent 90 degree pulses. All pulses were along *x* unless otherwise shown. The experimental parameters were; $d_1$=0.1 s, $d_2$=1.5 ms, $G_1$=7.2 G/cm, gradient pulse width=2.0 ms, $^{1}$H soft pulse width in (b)=2.5 s. Phase cycling: $\varphi_1$=*x, -x, -x, x, y, -y, -y, y*; $\varphi_r$= *x, x, -x, -x, y, y, -y, -y*.

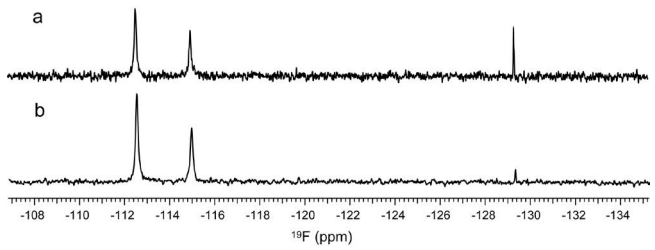


**Figure 3:** (a), (b) The $^{19}$F{$^{1}$H} STD spectra acquired using the pulse sequences shown in Figure 2a and 2b, respectively. The $^{1}$H resonance at 0.9 ppm was selectively excited. Double NMR tubes, which comprised a 3 mm tube containing 10 mM enoxacin and a 5 mm tube containing 5.0 mM diflunisal and 0.1 mM HSA, were used.

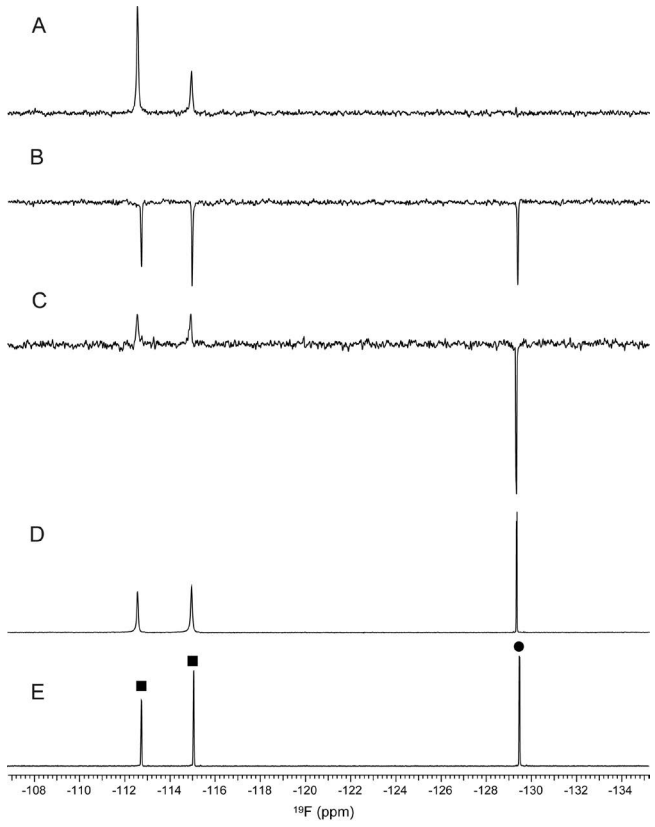


**Figure 4:** (a) The $^{19}$F{$^{1}$H} STD spectrum and (d) the reference $^{19}$F spectrum acquired using a sample solution of 4.0 mM diflunisal (■) and 4.0 mM enoxacin (●). (b) The $^{19}$F{$^{1}$H} STD spectra and (c) the reference $^{19}$F spectrum acquired using double NMR tubes, which comprised a 3 mm tube containing 10 mM enoxacin and a 5mm tube containing 5.0 mM diflunisal and 0.1 mM HSA. In (a) and (b), $^{1}$H resonance at 4.8 ppm was selectively excited.

## Conclusion

It was demonstrated that the WaterLOGSY type experiment with $^{19}$F detection was an effective method to selectively detect the fluorinated compounds bound to macromolecules. The $^{19}$F detection was advantageous in setting the NMR experiments, because suppression of the protein signals before acquisition using the $T_2$-filter was unnecessary and sample solutions containing 10% $^{2}H_2O$ can be used for the present experiments. Solvent exchange to 100% $^{2}H_2O$ often causes loss of the valuable samples. The proposed approach for discrimination of binding is expected to be a useful NMR-based screening method applicable to the fluorinated compounds.

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