

Quantitative Recovery Ordered (Q-ROSY) and Diffusion Ordered Spectroscopy using the Spoiler Recovery Approach

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Abstract. Combined PFG and T_1 methods for rapid acquisition of multiple scans of an NMR pulse sequence are presented. The methods apply initially two RF-pulses in combination with two magnetic field gradient pulses of opposite polarity, different strengths and different durations. The basic idea is to spoil any magnetization in any direction before letting the system recover to some degree of restoration of the thermal equilibrium magnetization. Thereafter any pulse sequence can be applied, and the next scan may be run immediately after the end of this spoiler pulse sequence. Thus one avoids the 5 times T_1 delay between each scan. The method has been verified at 11.7 Tesla correlating spectral information with T_1 or diffusion.

Keywords: relaxation, two dimensional correlation spectra, DOSY, ROSY

1. Introduction

Relaxation time and diffusion measurements are valuable tools when measuring NMR at low magnetic field with little or no spectral resolution. As the chemical shift is small or insignificant, the phases of the echo peak of the different components are coherent. Thus one may perform transverse relaxation time measurements in one go as in the CPMG sequence [1, 2] where thousands of data points may be acquired.

A measurement of the longitudinal relaxation time is a more time consuming task in order to acquire enough data points to do a proper data analysis [3]. This is because one gains only one data point per scan and has to wait the ordinary 5 times T_1 between each of them for thermal equilibrium to be established between each scan. Recently methods have been proposed for circumventing this waiting time [4-7]. One major drawback using these methods that establish a kind of saturation magnetization equilibrium is the application of a large number of pre-radiofrequency pulses, which may lead to unwanted coherences and poor signal to noise ratio for the wanted signal.

Instead of letting the magnetization reach thermal equilibrium before each scan, we apply a combination of RF-pulses and magnetic field gradients that aim at spoiling any magnetization in any direction. Then, using a waiting time equal to T_1 after the spoiling (SRD in figure 1), we have already regained 63% of the magnetization at thermal equilibrium. The waiting time between each scan is then reduced to practically nothing, and the total experimental time may be reduced by as much as 80% without any significant loss of signal to noise. In the following we will focus on describing the set-up for acquiring a rapid DOSY experiment and a quantitative T_1 experiment with spectral resolution (Q-ROSY). After performing the ordinary Fourier transform of the FID's we apply an Inverse Laplace transform of each frequency component in the spectra as a function of gradient strength (DOSY) or recovery delay (Q-ROSY). Thus we may in a two dimensional distribution get a

distribution of diffusion coefficients or T_1 in one direction whilst having spectral resolution along the other.

Using the proposed sequences and processing tool we have a rapid experimental set-up for studying mixtures of molecules and identifying resonances based on their chemical shift as well as their diffusion and relaxation behaviour. This includes studies of dimer formation and ligand – protein interactions. As will be shown in section 4 one important result of the proposed sequences is that whilst the DOSY returns the same diffusion coefficient from protons sitting on a macromolecule, the Q-ROSY may return different values of T_1 from the very same protons. This could be used as a probe to the shape of macromolecules in solutions, for example whether it is folded or not.

2. Theory

The conventional approach for measuring diffusion and or relaxation is to let the magnetization recover back to thermal equilibrium after application of a set of RF- and gradient pulses. Improvements have been made to reduce this acquisition time, and are based on saturating the magnetization to a steady state before applying the pulse sequences [4-7]. Our approach is different as we initially do not assume any particular state for the net nuclear magnetization. Regardless of the initial state we apply two 90 degree RF pulses in combination a bipolar pair of magnetic field gradients of arbitrary shape. This we call the spoiler recovery sequence [8]. In the following we will present two applications of the spoiler recovery at high resolution; the rapid DOSY and quantitative recovery ordered spectroscopy (Q-ROSY).

2.1. Rapid Diffusion Ordered Spectroscopy

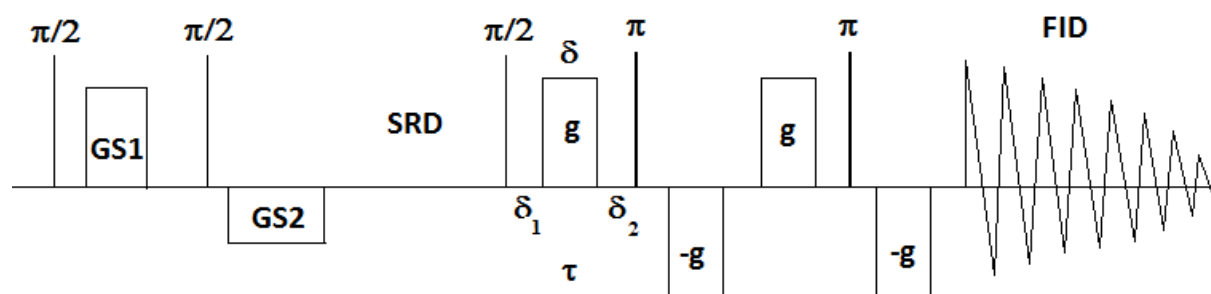


Figure 1 The rapid DOSY experiment

Figure 1 shows the rapid DOSY sequence, where the attenuation of the Fourier transformed FID for a multiexponential system at a given frequency, ν is written as

$$I_{(v)} = I_{0(v)} \sum_i \rho_i \left(1 - \exp\left(-\frac{\text{SRD}^i}{T_1^i}\right) \exp\left(-\gamma^2 4g^2 \delta^2 D^i \left(\frac{3}{2}\tau - \frac{\delta}{6}\right) - \frac{4\tau}{T_2^i}\right) \right) \quad (1)$$

Where γ is the gyromagnetic ratio, ρ_i is the weighting factor of region i , D^i is the diffusion coefficient, g is the applied gradient strength, $GS1$ and $GS2$ are the spoiler gradient pulses, δ_1 , δ_2 , δ and τ are time duration parameters, SRD is the spoiler recovery delay.

By fixing SRD and just incrementing the gradient strength we then get a dataset of FID's as a function of gradient strength. After a Fourier transform of the FID, the spectra is subjected to a one dimensional inverse Laplace routine [9], resulting in a distribution of diffusion coefficients for each frequency point in the spectra. Looking at equation 1 we see that by changing SRD we may get different contributions from components with different T_1 relaxation time. This can be used for solvent suppression as one usually wants to study macromolecules with rather short T_1 dissolved in some kind of solvent with much longer T_1 .

2.2. Quantitative Recovery Ordered Spectroscopy (Q-ROSY)

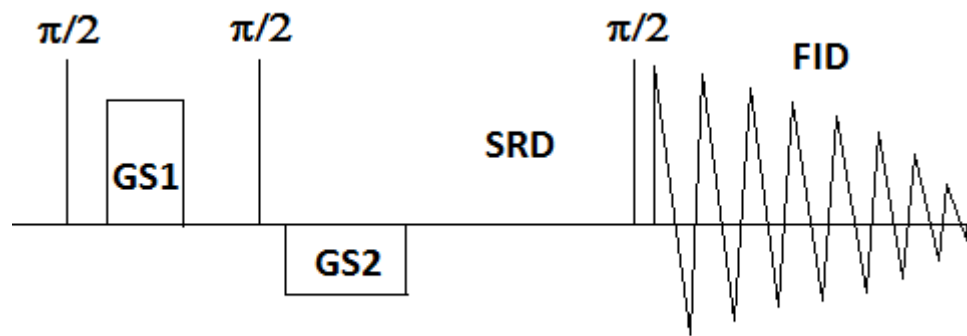


Figure 2 The Q-ROSY experiment.

In figure 2 we show the simplest sequence using the spoiler recovery sequence, only an extra 90 degree RF-pulse followed by acquisition of the FID. Again having Fourier transformed the FID of the monoexponential system, the attenuation at a given frequency, ν is written as

$$I(\nu) = I_0(\nu) \sum_i \rho_i \left(1 - \exp\left(-\frac{\text{SRD}}{T_i}\right)\right) \quad (2)$$

Compared to the standard inversion recovery sequence [3] there are some important advantages using the spoiler recovery sequence. First the 5 times T_I is avoided and the acquisition time is for each observation time (SRD) is number of scans times (SRD + acquisition time of FID). Thus the sequences with shortest SRD are recorded in a second or so while the only time consuming acquisitions are the ones when SRD approaches 5 times T_I . Secondly the signal does not start at a negative value, passing through the 0 or baseline where the signal is within the noise, and ending up at thermal equilibrium. In the spoiler recovery sequence the signal starts at zero with the shortest SRD time and grows up to thermal equilibrium as the SRD time increases. This makes it easier to analyze using 1D-ILT as the data set will not be as sensitive to offset values from the 0 baseline

3. Experimental

Here we present a set of sequences that uses the spoiler recovery sequence to reduce the acquisition time by approximately 80 % or more, without any significant loss of signal to noise. The high resolution measurements were performed on a Bruker 500 MHz Avance III instrument with 5 mm diffusion probe with actively shielded magnetic field gradients up to 18 T/m. Samples used were:

- 2mM Lysozyme in H₂O/D₂O 90/10 (11.7 Tesla)
- One asphaltene solutions (11.7 Tesla): The solution was prepared by dissolving asphaltenes in toluene-D₈ (99.6 atom %D, Aldrich) at a concentration of 1wt% and then magnetically stirred overnight.

4. Results and discussion

4.1 The rapid two dimensional experiments applied on a 2 mM lysozyme sample

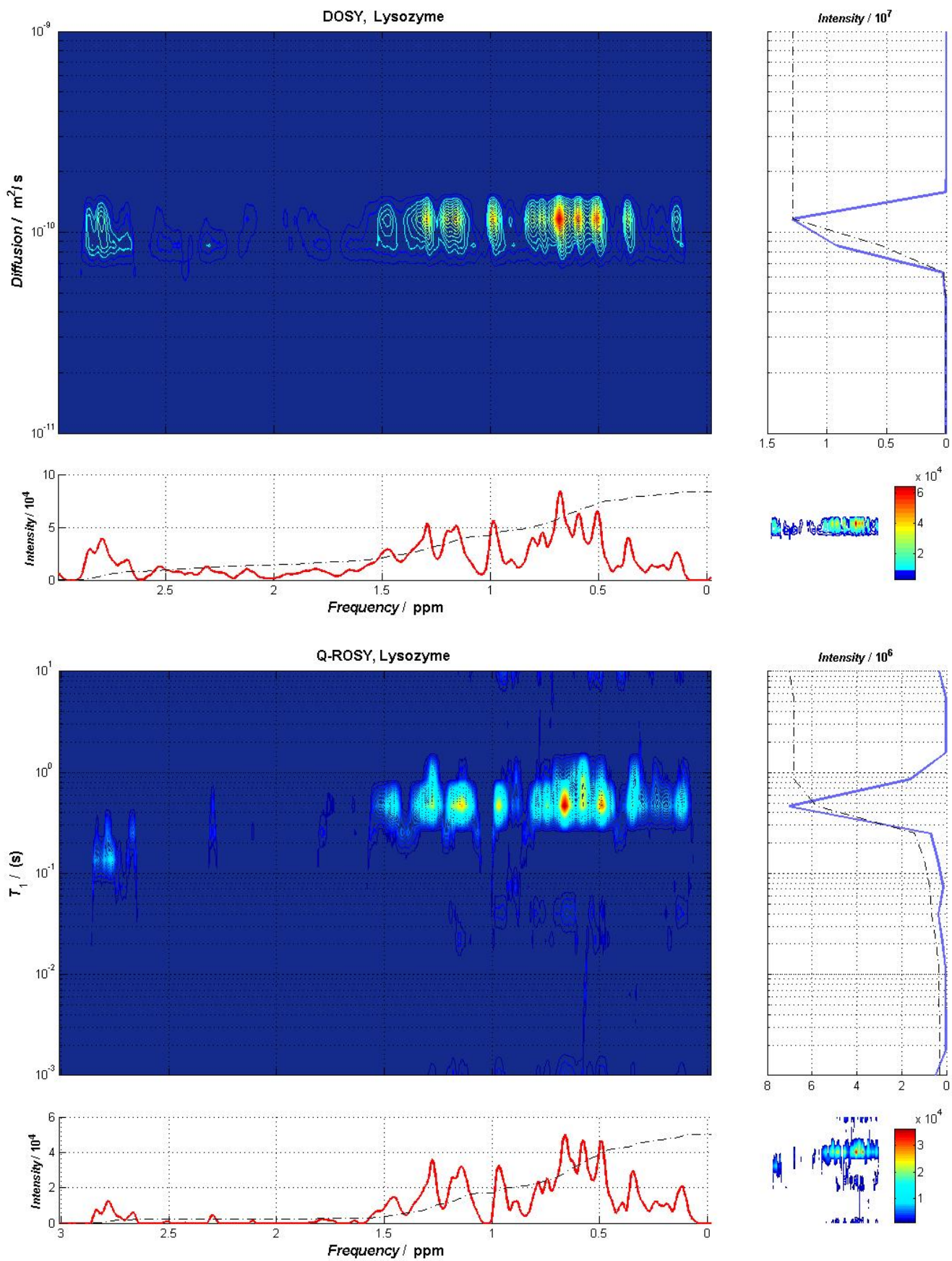


Figure 3 Rapid DOSY and Q-ROSY applied on Lysozyme

In figure 3 we see the DOSY and Q-ROSY Fourier - Inverse – Laplace – Transformed spectra (FILT) of lysozyme. As both 16 gradient strengths and SRD values were used, the maximum number of components along the diffusion and T_1 direction is 16. The grid in the ILT procedure is set to be between 10^{-9} m²/s and 10^{-11} m²/s for the DOSY and 10^1 s and 10^{-3} s for the Q-ROSY. Thus the resolution is such that it is basically pure luck to have monoexponential decay presented as a sharp peak along the D or T_1 direction. The monoexponential decay will usually be represented by a weighting between two points in the D or T_1 direction. As a single component value usually will lie between two such grid points, the sum of the two weighted grid points will result in monoexponential decay with the single component diffusion coefficient or relaxation time. That is why the diffusion distribution of the rapid DOSY of the lysozyme is rather broad compared to the signal along the frequency axis. The resolution along the D and T_1 direction may be improved by increasing the number of measuring points at the expense of increased experimental time. This may also increase the time it takes to process the data matrix. Currently we do process the 8192*16 data points into a FILT in 0.75 seconds using Intel Core i7 930 @ 4GHz. The weighting/smoothing of the data set for the ILT procedure is then fixed. While all lysozyme peaks have the same diffusion coefficient, we see that the region between 2.5 -3.0 ppm has a much shorter spin lattice relaxation time T_1 . This must be due to the way this group of protons are situated on the lysozyme molecule. We also see that the peak at ~0.6 ppm has a broader T_1 distribution than the adjacent peaks. It is likely that a larger number of data points (SRD delays) could have revealed a bimodal structure for some of the peaks in the lysozyme spectra.

4.2 The rapid 2 dimensional experiments applied on an Asphaltene sample

In figure 4 we show the FILT of the rapid DOSY and Q-ROSY on a diluted asphaltene sample. At ~2 ppm we do have a solvent signal while the rest is from the protons sitting on the asphaltene. The signal intensity of the solvent at 2 ppm is much weaker in the DOSY spectra because, as mentioned in section 2.1, a rather short duration of the spoiler recovery delay (SRD=500 ms) would reduce the intensity of the components with the longest T_1 .

The distribution of asphaltene shows to major peaks (0.8 and 1.3 ppm) and a broad band that covers the whole frequency range from 0.5 to 3.0 ppm. Compared to the lysozyme, the diffusion and T_1 distributions vary much more, over one order of magnitude. Whilst the lysozyme exhibit no significant variation in diffusion coefficient as function of frequency though indication of some variation in T_1 , we do see a significant variation of both diffusion and T_1 as a function of frequency in the asphaltene FILT. This is of course due to the fact that lysozyme is a well defined molecule with the same molecular size while the asphaltene is a conglomerate of hydrocarbons of different molecular sizes and structures. However, we do see a major cluster at 4.05 ppm with diffusion coefficient of $\sim 2.2 \cdot 10^{-10} \text{ m}^2/\text{s}$ and T_1 of ~ 800 ms. Then we have small clusters upwards and downwards that are manifested in the slightly multexponential decay of the bulk diffusion measurement presented on the very same sample by Sørland et.al [8].

The difference we see from measuring on lysozyme and asphaltene confirms the power of the use of rapid DOSY and Q-ROSY together. Not only can we get a fingerprint of the molecular sizes that are correlated to the diffusion coefficient through the Stoke-Einstein relation [10], but from the Q-ROSY we also get quantitative information and lattice bonding information from the T_1 values.

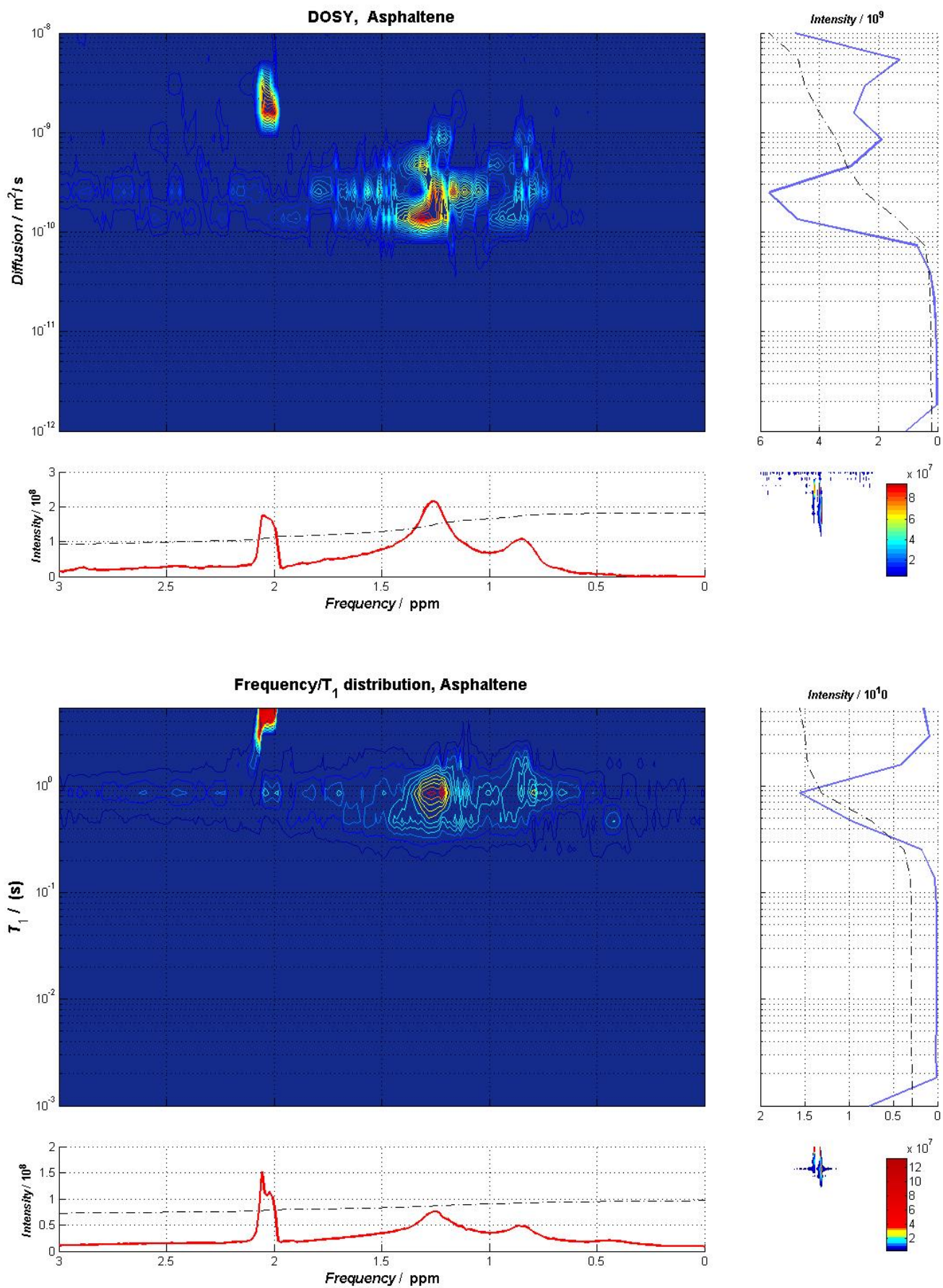


Figure 4 Rapid DOSY and Q-ROSY applied on asphaltene

5. Conclusion

The spoiler recovery has shown to be an important tool for reducing the total acquisition time for a two-dimensional NMR experiment. The combination of the spoiler recovery sequence with DOSY and ROSY provides better insight into the system being investigated.

6. References

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