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Communication

# Real-space imaging of macroscopic diffusion and slow flow by singlet tagging MRI

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ABSTRACT

Magnetic resonance imaging can be used to study motional processes such as flow and diffusion, but the accessible timescales are limited by longitudinal relaxation. The spatially selective conversion from magnetization to long-lived singlet order in designer molecules makes it possible to tag a region of interest for an extended period of time, of the order of several minutes. Here we exploit this concept of "singlet tagging" to monitor diffusion over a macroscopic scale as well as very slow flow.


## 1. Introduction

Beyond anatomical images, magnetic resonance can provide detailed information on motional processes, over a large range of temporal and spatial scales [1–3]. Many techniques rely on the concept of "time-of-flight" imaging, which consists of tagging a region of interest and tracking its subsequent evolution. Alternatively, various forms of motion can be characterized, sometimes quantitatively, with reciprocal-space (q-space) imaging techniques [3], which are applicable on spatial scales smaller than the accessible imaging resolution. The time scales that can be probed with magnetic resonance are, however, constrained by the longitudinal relaxation time, $T_1$. A region tagged through its magnetization will become indistinguishable from the rest of the sample within several periods of $T_1$. The study of slow processes thus requires general strategies to extend NMR timescales.

Long-lived states [4] allow spin magnetization to be stored for a considerably extended period of time, typically at least an order of magnitude longer than $T_1$ for the same molecule. Long-lived states act as repositories of polarization (and hyperpolarisation) that survive the presence of high magnetic fields and are insensitive to radiofrequency fields and field gradient pulses, unless these are played in a very selective manner [5]. This ability to selectively manipulate long-lived states offers the potential for a *smart probe* that can be activated on demand. To date, several strategies have been proposed to manipulate long-lived states [6–10], including highly selective approaches that can be applied in high field and therefore retain all the advantages of a smart probe [8,11]. Recently, we have demonstrated the possibility to convert magnetization to and from singlet order – the prototypical long-lived state formed by a spin pair – in a spatially selective manner, in the high field of an NMR/MRI magnet [12]. These techniques are the basis of "singlet tagging", which consists of converting a portion of a sample into long-lived singlet order, to be revealed at a later time or at a different position in space. Singlet tagging is analogous to spin labeling [13,14], with the advantage that the probe can be followed for a much longer time.

In this communication, we exploit the concept of singlet tagging to access extended timescales in MRI and monitor slow motional processes. For these experiments we make use of two recently synthesized molecules, capable of supporting singlet states with lifetimes of a few minutes. We first study an example of very slow flow and, subsequently, show how diffusion can be monitored in real space and over a macroscopic distance, once molecules can be tracked for several minutes.

## 2. Methods

### 2.1. Imaging experiments

All experiments were run on a Bruker 11.7 T Avance III NMR instrument. Two custom molecules (Scheme 1) were used for demonstration purposes.

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Flow imaging experiments were carried out on a 0.8 M sample of 1,2,3,4,5,6,8-heptakis(methoxy-$d_3$)-7-((propan-2-yl-$d_7$)oxy)-4a,8a-$^{13}C_2$-naphthalene [15] (Scheme 1**I**) dissolved in $(CD_3)_2CO$. The longitudinal relaxation decay constant of this sample was measured with a standard saturation-recovery experiment and is $T_1 = 9 \pm 0.1$ s. The singlet order decay constant for the same sample was measured as described in Ref. [11] and is $T_S = 260 \pm 16$ s, at 11.7 T and in an undegassed solution (estimated $[O_2] \sim 2$ mM at 293.15 K and $PO_2 = 19$ kPa). To achieve stationary flow the setup shown in Fig. 1 was used. It consists of a glass chamber (20 mm long, 5 mm OD, 4.2 mm ID) connected through a PTFE hose (0.3 mm ID) to a 2 ml injection syringe and a 2 ml recovery syringe (Fig. 1). The injection syringe is mounted on a syringe pump (KD Scientific Inc., USA), which can provide flow rates of up to 120 μl/min for the chosen syringe. The glass chamber sits in the MRI probe. The chamber volume is 280 μl while the *dead* volume of the connection hose is 200 μl. Prior to flow experiments the injection syringe, the glass chamber and the connection hose were filled with a solution of **I**.

Diffusion imaging experiments were carried out on a 1 M sample of 1-(ethyl-d5),4-(propyl-d7)(*Z*)-but-2-enedioate [12] (Scheme 1**II**) dissolved in $CD_3CN$. The sample was degassed by 4 pump–thaw cycles (to remove paramagnetic oxygen and therefore increase $T_1$ and $T_S$) and sealed into a 5 mm OD J-Young valve NMR tube. The longitudinal relaxation decay constant of this sample is $T_1 = 19.6 \pm 0.2$ s; its singlet order decay constant is $T_S = 360 \pm 30$ s.

All MRI experiments were performed on a micro-imaging probe coupled to a gradient system delivering up to 150 G/cm, using a 10 mm $^1$H coil for diffusion measurements and a 10 mm $^1$H/$^{13}$C coil for flow measurements. 2D images were obtained with a RARE MRI pulse sequence [16]. Vendor-provided sinc-3 pulses with duration of 1 ms were used to select sagittal and axial slices in the sM2S (Fig. 2b) for the macroscopic diffusion and slow flow experiments, respectively. Custom Paravision (Bruker, Billerica, MA) pulse sequences were used and the experimental data were processed with custom Mathematica (Wolfram Research Inc., Champaign, IL) routines.

### 2.2. Simulation of diffusion

Numerical simulations of diffusion were performed with Mathematica. A configuration of 100,000 molecules, initially confined in a sagittal slice of 0.9 mm thickness and 20 mm height was allowed to undergo random walk diffusion to reach a final configuration after a diffusion time of 10 μs, 1, 2, 3 and 5 min, in separate runs. Diffusion was confined within a cylinder of 4.2 mm diameter and 20 mm height that mimic the sample used in the diffusion experiments below. Pseudo-images were obtained as 2D arrays by counting the number of molecules in each voxel, with voxel geometry chosen to match the experimental one. Simulated images include randomly generated noise to match the experimental signal-to-noise ratio and an exponential decay of intensity, with a decay rate constant of 360 s, to account for singlet relaxation.

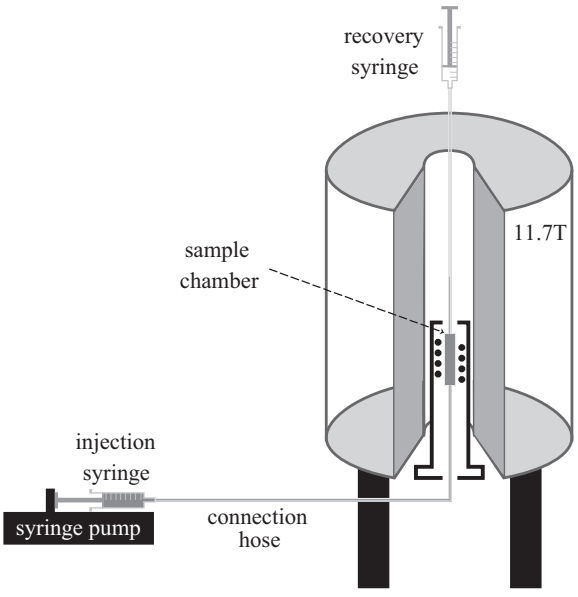
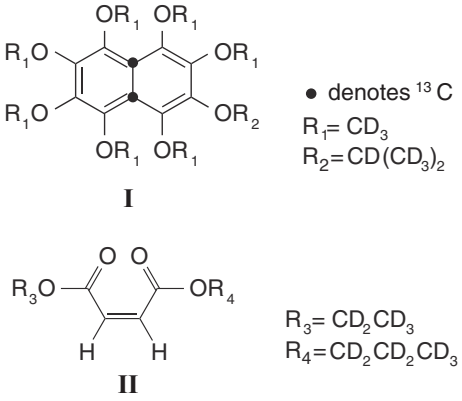



**Fig. 1.** Equipment used for imaging of slow flow.




**Scheme 1.** Molecular schemes of (**I**) 1,2,3,4,5,6,8-heptakis(methoxy-$d_3$)-7-((propan-2-yl-$d_7$)oxy)-4a,8a-$^{13}C_2$-naphthalene and (**II**) 1-(Ethyl-$d_5$) 4-(propyl-$d_7$)(*Z*)-but-2-enedioate.

## 3. Results and discussion

### 3.1. Singlet tagging

Singlet tagging refers to the spatially selective conversion from magnetization to long-lived singlet order (sM2S) in a solution of singlet-bearing molecules [12]. The pulse sequence used for singlet tagging experiments is shown in Fig. 2a. During the sM2S block, longitudinal magnetization is converted into singlet order only in a selected slice of the sample – the geometry of the selected slice is chosen through the parameters of the selective pulses and field gradients. After creation of singlet order, a variable time interval $t$ is left, during which the tagged molecules may move. Because of the long lifetime of singlet states in suitable molecules such as **I** ($T_S \sim 260$ s) and **II** ($T_S \sim 360$ s), $t$ can be up to several minutes and still yield images with suitable signal-to-noise ratio. After the delay $t$, a singlet filter [17] is used to destroy magnetization or any term other than singlet order (up to rank 3) that may have been created during $t$. A non-selective singlet-to-magnetization sequence (Fig. 2c) is run to reconvert singlet order into longitudinal magnetization. At this point, the spatial configuration of the tagged molecules can be interrogated by any suitable imaging technique. Here we have used a single-shot version of the RARE [16] pulse sequence, which provides both high sensitivity and a high robustness against field inhomogeneity. Although RARE is not typically used as a single-shot technique, the long transverse relaxation times of the two compounds studied here are compatible with very long echo times.

### 3.2. Slow flow imaging

The singlet tagging approach can be used to study slow flow with time-of-flight imaging techniques. A stationary flow was obtained here with the equipment shown in Fig. 1 where the flow