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TITLE:

Measuring the Spin-Lattice Relaxation Magnetic Field Dependence of Hyperpolarized [1-^{13}C]pyruvate

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SUMMARY:
We present a protocol to measure the magnetic field dependence of the spin-lattice relaxation time of ^{13}C-enriched compounds, hyperpolarized by means of dynamic nuclear polarization, using fast field-cycled relaxometry. Specifically, we have demonstrated this with [1-^{13}C]pyruvate, but the protocol could be extended to other hyperpolarized substrates.

ABSTRACT:
The fundamental limit to *in vivo* imaging applications of hyperpolarized ^{13}C-enriched compounds is their finite spin-lattice relaxation times. Various factors affect the relaxation rates, such as buffer composition, solution pH, temperature, and magnetic field. In this last regard, the spin-lattice relaxation time can be measured at clinical field strengths, but at lower fields, where these compounds are dispensed from the polarizer and transported to the MRI, the relaxation is even faster and difficult to measure. To have a better understanding of the amount of magnetization lost during transport, we used fast field-cycling relaxometry, with magnetic resonance detection of ^{13}C nuclei at ~0.75 T, to measure the nuclear magnetic resonance dispersion of the spin-lattice relaxation time of hyperpolarized [1-^{13}C]pyruvate. Dissolution dynamic nuclear polarization was used to produce hyperpolarized samples of pyruvate at a concentration of 80 mmol/L and physiological pH (~7.8). These solutions were rapidly transferred to a fast field-cycling relaxometer so that relaxation of the sample magnetization could be measured as a function of

time using a calibrated small flip angle (3°–5°). To map the T_1 dispersion of the C-1 of pyruvate, we recorded data for different relaxation fields ranging between 0.237 mT and 0.705 T. With this information, we determined an empirical equation to estimate the spin-lattice relaxation of the hyperpolarized substrate within the mentioned range of magnetic fields. These results can be used to predict the amount of magnetization lost during transport and to improve experimental designs to minimize signal loss.

INTRODUCTION:

Magnetic resonance spectroscopic imaging (MRSI) can produce spatial maps of metabolites detected by spectroscopic imaging, but its practical use is often limited by its relatively low sensitivity. This low sensitivity of in vivo magnetic resonance imaging and spectroscopy methods stems from the small degree of nuclear magnetization achievable at body temperatures and reasonable magnetic field strengths. However, this limitation can be overcome by the use of dynamic nuclear polarization (DNP) to greatly enhance the in vitro magnetization of liquid substrates, which are subsequently injected to probe in vivo metabolism using MRSI[1-4]. DNP is capable of enhancing the magnetization of most nuclei with non-zero nuclear spin and has been used to increase in vivo MRSI sensitivity of ^{13}C-enriched compounds such as pyruvate[5,6], bicarbonate[7,8], fumarate[9], lactate[10], glutamine[11], and others by more than four orders of magnitude[12]. Its applications include imaging of vascular disease[13-15], organ perfusion[13,16-18], cancer detection[1,19-22], tumor staging[23,24], and quantification of therapeutic response[2,6,23-26].

Slow spin-lattice relaxation is essential for in vivo detection with MRSI. Spin-lattice relaxation times (T_1s) on the order of tens of seconds are possible for nuclei with low gyromagnetic ratios within small molecules in solution. Several physical factors influence the transfer of energy between a nuclear spin transition and its environment (lattice) leading to relaxation, including the magnetic field strength, temperature, and molecular conformation[27]. Dipolar relaxation is reduced in molecules for carbon positions with no protons directly attached, and deuteration of dissolution media can further reduce intermolecular dipolar relaxation. Unfortunately, deuterated solvents have limited abilities to extend in vivo relaxation. Increased relaxation of carbonyls or carboxylic acids (such as pyruvate) can occur at high magnetic field strengths due to chemical shift anisotropy. The presence of paramagnetic impurities from the fluid path during dissolution after polarization can cause rapid relaxation and need to be avoided or eliminated using chelators.

Very little data exist for the relaxation of ^{13}C-containing compounds at low fields, where spin-lattice relaxation could be significantly faster. However, it is important to measure T_1 at low fields to understand relaxation during preparation of the agent used for in vivo imaging, since the hyperpolarized contrast agents are usually dispensed from the DNP apparatus near or at the earth's field. Additional physical factors such as ^{13}C-enriched substrate concentration, solution pH, buffers and temperature also influence relaxation, and consequently have an effect on the formulation of the agent. All these factors are essential in the determination of key parameters in optimizing the DNP dissolution process, and the calculation of the magnitude of signal loss that occurs in transportation of the sample from the DNP apparatus to the imaging magnet.

Nuclear magnetic resonance dispersion (NMRD) measurements, i.e., T_1 measurements, as a function of magnetic field are typically acquired using an NMR spectrometer. To acquire these measurements, a shuttling method could be used where the sample is first shuttled out of the spectrometer to relax at some field determined by its position in the fringe field of the magnet[28-30] and then rapidly transferred back into the NMR magnet to measure its remaining magnetization. By repeating this process at the same point in the magnetic field but with increasing periods of relaxation, a relaxation curve can be obtained, which can then be analyzed to estimate T_1.

We use an alternative technique known as fast field-cycling relaxometry[31-33] to acquire our NMRD data. We have modified a commercial field-cycling relaxometer (see **Table of Materials**), for T_1 measurements of solutions containing hyperpolarized ^{13}C nuclei. Compared with the shuttle method, field-cycling enables this relaxometer to systematically acquire NMRD data over a smaller range of magnetic fields (0.25 mT to 1 T). This is accomplished by rapidly changing the magnetic field itself, not the sample location in the magnetic field. Therefore, a sample can be magnetized at a high field strength, "relaxed" at a lower field strength, and then measured by acquisition of a free-induction-decay at a fixed field (and Larmor frequency) to maximize signal. This means that the sample temperature can be controlled during the measurement, and the NMR probe does not need to be tuned at each relaxation field promoting automatic acquisition over the entire magnetic field range.

Focusing our efforts to the effects of dispensing and transporting the hyperpolarized solutions at low magnetic fields, this work presents a detailed methodology to measure the spin-lattice relaxation time of hyperpolarized ^{13}C-pyruvate using fast field-cycling relaxometry for magnetic fields in the range of 0.237 mT to 0.705 T. The main results of using this methodology have been previously presented for [1-^{13}C]pyruvate[34] and ^{13}C-enriched sodium and cesium bicarbonate[35] where other factors such as radical concentration and dissolution pH have also been studied.

PROTOCOL:

1. Sample Preparation

NOTE: Steps 1.1–1.8 are performed just once

1.1. Prepare 1 mL of stock ^{13}C-enriched pyruvic acid solution, widely used for *in vivo* research[1,2,5,6], consisting of 15-mmol/L of triarylmethyl radical dissolved in [1-^{13}C]pyruvic acid (see **Table of Materials**). Aliquots from this stock solution will be used for the samples that will be individually polarized and subsequently undergo relaxometry at different magnetic fields. A representation of the [1-^{13}C]pyruvic acid molecule is shown in **Figure 1**.

1.2. On the dynamic nuclear polarizer software interface (see **Table of Materials**), click on the **Cooldown** button to lower the temperature of the variable temperature insert (VTI) to 1.4 K.

1.3. Once the DNP has reached the desired temperature, load 10 µL of the stock solution in a sample cup, open the turret doors and insert the cup into the VTI using an insertion wand specifically designed for this task.

1.4. After that, quickly extract the wand and make sure the cup is released. Then close the turret doors and continue with the following steps while the temperature of the VTI goes back to 1.4 K.

1.5. Prepare the DNP to run a microwave sweep in order to find the optimal RF frequency for hyperpolarization of the stock solution.

1.5.1. On the computer controlling the spectrometer (part of the DNP system), establish the communication between the spectrometer and the DNP control software by double-clicking on HyperTerminal icon, previously configured with the appropriate serial communication parameters.

1.5.2. Once the communication is established, launch the RINMR software, type in its command line**.HYPERSENSENMR**, and then press **enter**.

1.5.3. After that, a new window will be shown on the screen and on it type the number one (1) in the **Configuration Number** field. Then, click on the **Select Configuration** button.

1.5.4. Click on the button **Do microwave sweep**. A small window with a descending counter of seconds will be launched indicating that the spectrometer is ready and it will be waiting for periodic trigger signals, coming from the DNP control software, to sample the polarization.

1.5.5. On the DNP control software, select the **Calibrate** tab and click on the **Generate** button.

1.5.6. Using the calibration setup window, enter the following information: Start Frequency = 94.117 GHz, End Frequency = 94.137 GHz, Step Size = 1 MHz, Step Duration = 300 s, Power = 50 mW, Liquid Helium Level = 65%, and Temperature = 1.4 K.

1.5.7. Click on the button **Generate**, which will close the setup window and return to the **Calibrate** tab that will display the number of steps and the time required to perform the desired microwave sweep.

1.5.8. Once the desired VTI temperature is achieved, click the **Enable** button and then **Start** to initialize the microwave sweep process.

1.6. At the end of the microwave sweep, recover the sample and record the optimal frequency where the maximum polarization is achieved. This optimal frequency is defined as the polarization frequency that provides the maximum polarization as shown in **Figure 2**. This frequency will be used for hyperpolarizing all the aliquots obtained from that specific stock solution of pyruvic acid.

1.7. Prepare 250 mL of stock dissolution medium using a solution of 40-mmol/L Tris base, 50 mmol/L of sodium chloride, and 80-mmol/L sodium hydroxide in de-ionized water. Add

ethylenediaminetetraacetic acid (EDTA) at a concentration of 100 mg/L to sequester any metal ion contamination. Similarly to the pyruvic acid stock solution, this dissolution medium will be used for all the different samples that will be polarized. Refer to the **Table of Materials** for more specific details regarding the chemicals used.

1.8. Also, prepare 500 mL of stock cleaning solution consisting of 100 mg/L EDTA dissolved in deionized water. Approximately 10 mL of this cleaning solution is used after each polarization to clean the dissolution path of the DNP.

NOTE: Steps 1.9–1.27 are performed for each individual sample.

1.9. Cool the DNP apparatus to 1.4 K in preparation of hyperpolarizing a [1-^{13}C]pyruvic acid sample by pressing the **Cooldown** button in the DNP main window.

1.10. If the software used for the spectrometer is already active with configuration **1** selected, proceed with the following steps. Otherwise, perform steps 1.5.1 to 1.5.3 and then continue with the following steps.

1.11. After verifying that configuration **1** is selected in the window controlling the DNP's spectrometer, click on the **Solid Build Up** button.

1.12. Enter the file name SSBuilupXXX, where "XXX" is a number in the sequence of files stored with build-up data. This number is automatically incremented by the software. Then click **OK**. Similarly to the microwave sweep case, a small window with a descending counter of seconds will be launched indicating that the spectrometer is ready and it will be waiting for periodic trigger signals, coming from the DNP control software, to sample the polarization.

1.13. Using the pyruvic acid - OX063 stock solution prepared in step 1.1, weigh out 30 mg in a sample cup.

1.14. When the desired VTI temperature is achieved (1.4 K) click on **Insert Sample**, then select **Normal Sample** and then click on **Next**. Following the safety precautions displayed on the screen, insert the cup in the cold DNP apparatus, using a long wand specifically designed for this task.

1.15. Once the cup is inserted, the wand removed, and the DNP doors closed, click **Next** and then **Finish**. At that point the hyperpolarizer system lowers the sample cup to the irradiation chamber partially filled (65%) with liquid helium.

1.16. Wait until the temperature has returned to 1.4K and then click on the **Polarize Sample** button.

1.17. In the new pop up window, set the frequency value to that obtained from the microwave sweep in step 1.6. In the same window, also set the power to 50 mW and the sampling time to 300 s. Click on **Next**, check the **Enable Build-up Monitoring** box, and then click on **Finish**.

NOTE: Once the polarization is started, the DNP control software generates trigger signals every 300 s to instruct the spectrometer to sample the polarization using a small tip angle. That way, the spectrometer software adds a sample point to a solid-state magnetization curve, now displayed in both the spectrometer software and in the DNP control software under the tab **Polarization Build-Up**. After the 4th sample and every sample after that, the spectrometer software fits the curve to an exponential growth function of the form:

$$S = A * \exp(-t/T_p) + y_0$$

where A is the polarization amplitude, in arbitrary units, t is the sampling time, T_p is the polarization time constant (both in seconds), and y_0 is an offset. Based on the fitted parameters, the software also calculates the percentage polarization achieved up to that point in time, which is also displayed in the DNP's Polarization Status tab.

1.18. Polarize until the build-up of the solid-state magnetization reaches at least 95% of maximum (approximately one hour).

1.19. While the sample is polarizing, prepare the Fast-Field-Cycling Relaxometer as explained in Section 2 below.

1.20. When the desired polarization is achieved, click on **Run Dissolution** and under **Method**, select **Pyruvic Acid Test**. Then, click on **Next**.

1.21. Following the instructions on the screen, open the DNP turret doors and load the heating and pressurizing chamber at the top of the apparatus with ~ 4.55 mL of the dissolution medium prepared in section 1.5 to produce a concentration of 80-mmol/L pyruvate upon dissolution at a pH of ~7.75 and temperature of ~37 °C.

1.22. Position the recovering wand in the right position, close the turret doors, and at the computer click on **Next** and then on **Finish**. At that point the dissolution media will be superheated until the pressure reaches 10 bar.

1.23. Once the 10 bar pressure is attained, the frozen and hyperpolarized pyruvate is automatically lifted from the liquid helium bath, quickly mixed, and thawed with the superheated dissolution media and ejected through a capillary tubing into a pear-shaped flask. While the hyperpolarized pyruvate/dissolution media mixture is ejected, constantly swirl the flask to ensure a homogeneous mixture.

1.24. When all the mixture has been ejected, quickly draw 1.1 mL of the liquid into a syringe, transfer to a pre-warmed (37 °C) 10-mm-diameter NMR tube, and rapidly transport to the field-cycling relaxometer (See step 2.2.12).

1.25. Dispense the remaining aliquot of every pyruvate dissolution into a 0.55-T benchtop NMR spectrometer (see **Table of Materials**) to check for possible systematic experimental effects.

1.26. Immediately clean DNP fluid path using clean dissolution medium followed by ethanol. Blow helium gas through fluid path to remove remaining cleaning fluids and purge path of oxygen. Clean all glassware.

1.27. After each measurement, record the pH of samples from both the bench top spectrometer and the field-cycling relaxometer.

NOTE: Each T_1 measurement is a separate hyperpolarized dissolution from the DNP apparatus, so care is required to assure measurement-to-measurement reproducibility of the sample composition. This is accomplished by weighing all agents and solvents with a precision of 0.1 mg to assure accurate and reproducible preparation of the final hyperpolarized solutions.

2. Relaxometry

NOTE Please refer to **Table 1** for a better understanding of the selection and use of the different parameters described in the following steps. Prior to dissolution, the relaxometer flip angle must be calculate and the relaxometer must be setup and ready for measurement of the hyperpolarized solution (see below).

2.1. Flip-angle calibration

2.1.1. Prepare 1 mL of neat [1-^{13}C]pyruvic acid in an NMR tube and add a gadolinium contrast agent to reduce the T_1 of the ^{13}C nuclei to a value of less than 200 ms but more than 50 ms.

2.1.2. Seal the NMR tube so it can be used multiple times as a calibration standard.

2.1.3. Using the depth gauge of the relaxometer, set the depth of insertion of the NMR tube to the proper height to ensure the sample will be located at the center of the relaxometer RF coil.

2.1.4. Mark the insertion depth of the ^{13}C pyruvate calibration standard with adhesive tape to ensure repeatability.

2.1.5. Place the depth stopper on the NMR tube to the position indicated by the tape and insert this calibration standard into the bore of the field-cycling relaxometer. Use a weight to keep the NMR tube in position.

2.1.6. Open the instrument air valve and from the relaxometer front panel set the temperature controller to 37 °C. That will maintain the temperature of the sample at 37°C (± 0.5°C) using heated air during the experiment.

2.1.7. Setup the field-cycling relaxometer hardware to acquire ^{13}C nuclei signals. That includes installing and energizing the external shim coil (see **Table of Materials**), tuning and matching the RF coil to 8 MHz (~0.75 T for ^{13}C nuclei), and using the appropriate λ/4 cable.

2.1.8. In the instrument software, perform the following steps:

2.1.8.1. Select the **Main par** tab

2.1.8.2. Click on the cell next to the label **Experiment** and scroll down in the pop-up window to select the pulse sequence "13CANGLE.FFC ".

2.1.8.3. Set the following acquisition parameters: RFA = 5; SWT = 0.005, RD = 0.5, BPOL = 30 MHz, TPOL = 0.5.

2.1.8.4. Select the **Acq. par** tab and then select the **Basic** sub-tab.

2.1.8.5. Click on the cell next to the label **Nucleus** and scroll down in the pop-up window to select **13C**.

2.1.8.6. Then, set the following parameters: SF = 8 MHz, SW = 1000000, BS = 652, FLTR = 100000, MS = 32.

2.1.8.7. Select the **Conf** sub-tab.

2.1.8.8. Set the following parameters: RINH = 25, ACQD = 25.

2.1.8.9. Select the **nDim** sub-tab

2.1.8.10. Set NBLK = 32, BINI = 2, BEND = 62.

2.1.8.11. Select the **Evaluation** tab and then the **Parameters** sub-tab.

2.1.8.12. Set the following parameters: EWIP = 10, EWEP = 128, EWIB = 1, EWEB = 32.

2.1.8.13. Then, click the **Start Acquisition** icon to run the pulse sequence.

2.1.9. Once the acquisition is finished, save the data, select the **Evaluation dialog** icon and from the analysis menu select **WAM Window: Absolute Magnitude**. Then select **Report Sheet**, **Graphs** and **Export File** and finally click on **Execute**.

2.1.10. In the Report window find the RF pulse width that provides the maximum amplitude and fine-tune the value with the help of the cursor in the displayed graph, which is similar to the plots shown at the bottom row of **Figure 3**. This pulse width will be used for the parameter PW90 of the following experiments.

2.1.11. Click the **F1** icon to adjust the frequency shift of the relaxometer.

NOTE: **WAM Window: Absolute Magnitude** is a procedure to integrate the magnitude of a single or a sequence of free-induction decay acquisitions (FIDs) from the point defined by **EWIP** to the point specified by **EWEP** and from the block defined by **EWIB** to the block specified by **EWEB**.

2.2. T_1-Measurements

2.2.1. Make sure the external shim coil is installed and energized.

2.2.2. In the instrument software perform the following steps:

2.2.2.1. Select the **Main par** tab

2.2.2.2. Click on the cell next to the label **Experiment** and scroll down in the pop-up window to select the pulse sequence **HPUB/S**, which is shown in **Figure 4**.

2.2.2.3. Set the following acquisition parameters: RFA = 25, T1MX = values between 3 and 5; SWT = 0.2, RD = 0, BRLX = Desired relaxation field in MHz (proton Larmor frequency).

2.2.2.4. Select the **Acq. par** tab and then select the **Basic** sub-tab.

2.2.2.5. Click on the cell next to the label **Nucleus** and scroll down in the pop-up window to select **13C**.

2.2.2.6. Then, set the following parameters: SF = 8 MHz, SW = 1000000, BS = 652, FLTR = 50000.

2.2.2.7. Select the **Conf** sub-tab.

2.2.2.8. Set the following parameters: PW90 equal to the value found in step 2.1.10, RINH = 25, ACQD = 25.

2.2.2.9. Select the **Puls** sub-tab and set PW = 5.

2.2.2.10. Select the **nDim** sub-tab and set NBLK = 100.

2.2.2.11. Wait and get ready to receive the hyperpolarized solution to initiate the data acquisition.

2.2.2.12. Immediately before inserting the sample into the relaxometer, manually start the pulse sequence from the console, to avoid inserting the sample into a null magnetic field. For this reason, it is important to ignore the first Free Induction Decay (FID) during the data analysis.

2.2.2.13. Once the acquisition is done, save the data by clicking the **Save** button.

2.2.3. Using the analysis software, integrate the magnitude of each FID signal to produce a data series comprised of sample magnetization as a function of time.

2.2.4. Extract the spin-lattice relaxation time from a three-parameter exponential model using a standard non-linear least-squares fitting algorithm implemented in a commercial analytical software (see **Table of Materials**) assuming even weighting for all data:

$$signal = Acos^{(n-1)}(\alpha)e^{-\frac{nT_R}{T_1}} + y_0$$

where A is the initial signal amplitude (y-intercept), T_1 is the spin-lattice relaxation time, T_R is the repetition time, which is a known value, y_0 is the signal offset, and $cos^{(n-1)}(\alpha)$ is a correction for loss of longitudinal magnetization at the nth measurement for a flip angle, α.

REPRESENTATIVE RESULTS:

Figure 2 presents an example of a high-resolution full-range microwave sweep for pyruvic acid. For the presented case, that optimal microwave frequency corresponds to 94.128 GHz, highlighted in the figure insert. Our DNP system can normally work in the range of 93.750 GHz to 94.241 GHz with step size of 1 MHz, polarization time of up to 600 s, and power of up to 100 mW. A full range of frequencies is investigated only for novel substrates. However, based on previous experience with ^{13}C-pyruvic acid, we expect the optimal frequency to be around 94.127 GHz. Therefore, a scan range between 94.117 GHz to 94.137 GHz, with a step size of 1 MHz and a sampling time of 300 s with 50 mW of power, are typically used.

The left column of **Figure 3** presents the results for the tip angle calibration for [1-^{13}C]pyruvic acid, which involves acquisition of a series of signal measurements as a function of a linearly varying RF pulse durations to determine the pulse width corresponding to a flip angle of 90° and 180° for ^{13}C nuclei. The pulse width that provides the maximum amplitude corresponds to a flip angle of 90° and the zero crossing corresponds to a flip angle of 180°. The relationship between the two pulse widths should be a factor of two.

The acquisition parameters for the ^{13}C tip angle calibration shown above may require some adjustments depending on the transmit power of the field-cycle relaxometer, the T_1 of the sample, and the noise characteristic of the system. Some trial and error may be needed as well to properly find the 90° and 180° without the effects of stimulated echoes, amplifier saturation, and poor SNR.

This procedure, although accurate, is normally time consuming because the poor SNR of thermally polarized ^{13}C compounds requires many averages. An alternative and faster method involves calibrating the flip angle with a gadolinium-doped ^{1}H phantom and scaling the duration of the 90° RF pulse for ^{13}C by multiplying the duration of the 90°-^{1}H RF pulse by the ratio of the gyromagnetic ratios of ^{1}H/^{13}C, which corresponds to a factor of 3.976. For this case, the standard acquisition parameters should be: EXP = ANGLE.FFC, NUC = ^{1}H, TPOL = 0.1 s, BPOL = 30 MHz, SWT = 0.005, BINI = 0 µs, BEND = 15.5 µs, NBLK = 32, MS = 1, RFA = 25, RD = 0.1 s, BS = 652, SW = 1 MHz, FLTR = 100 KHz, SF = 8, RINH = 25, ACQD = 25, EWIP = 10, EWEP = 512, EWIB = 1, and EWEB = 32. The results for this alternative method are shown in the right column of **Figure 3**. As a comparison, for the presented cases, the total acquisition time for tip angle calibration for ^{13}C was 13.5 minutes whereas for ^{1}H was 7.1 seconds.

Figure 5 illustrates the typical series of decaying FIDs as the hyperpolarized magnetization is sampled. Each T_1 measurement at a given B_{RLX} is a separate hyperpolarized dissolution from the DNP apparatus. For this particular case, the relaxation field (B_{Relax}) was 0.2916 mT, with a

repetition time of 3.4 s and a flip angle of 5°. All sample temperatures were controlled to 37 °C (±0.5 °C).

Figure 6 presents the relaxation curve for hyperpolarized [1-^{13}C]pyruvate obtained from the data of the previous figure. Each blue point on the curve represents the area under an FID. The T_1 value (53.9 $\pm$ 0.6s) was obtained by a non-linear least-squares fit of the signal equation to the decay curve data, which included the effects of the flip angle used for excitation. The goodness of fit was assessed by computing the R^2 value (0.9995), assuming even weighting of the data points. Fitting residuals (data-fit) are shown as open triangles.

Figure 7 presents the T_1 results for all 26 measurements over a range of 0.237 mT and 0.705 T at 37 °C (±0.5 °C). The T_1 had an average fitting uncertainty of ±0.33 s for all the results. Analysis of the scatter of measurements repeated at a particular relaxation field yielded an experimental reproducibility several times larger than the statistical uncertainty quoted above, with a T_1 of 1.91 s. An uncertainty of 2.24 s was conservatively assigned for all T_1 measurements calculated as the sum of the two uncertainties quoted above. The T_1-dispersion data are well characterized by the empirical formula T_1 = (3.74 ± 0.52) x $\log_{10}(B_{Relax})$ + (63.0 ± 1.2) s; where B_{Relax} is the relaxation field measured in Tesla. The uncertainties for the fitted parameters represent one standard deviation. The solid line on **Figure 7** represents the formula along with the dashed lines representing the 95% confidence bands. pHs for these samples ranged from 7.63 to 7.93, with an average pH of 7.75 and a standard deviation of 0.09. Analysis of the results showed that the relaxation time for the C-1 nucleus is ~ 46.9 s at earth's magnetic field (0.05 mT) compared with ~ 65 s at 3 T, which represents a decrease of 28%.

FIGURE CAPTIONS:

Figure 1: [1-^{13}C]pyruvic acid molecule.

Figure 2: Full-range microwave sweep and zoom-in section showing the optimal polarization frequency.

Figure 3: Tip angle calibration for ^{13}C (left) and ^{1}H (right) samples.

Figure 4: Field-cycled pulse sequence (HPUB/S) to measure the T_1-relaxation time of a hyperpolarized sample at a particular relaxation field (B_{RLX}).

Figure 5: Sequence of FIDs obtained with the HPUB/S pulse sequence.

Figure 6: Relaxation signal (blue dots), curve fitting (red line), and fitting error (open triangles) obtained from the sequence of FIDs presented in Figure 5. This figure has been modified with permission from Chattergoon et al. 2013[34].

Figure 7: NMRD profile of hyperpolarized [1-^{13}C]pyruvic acid at low magnetic fields. This figure has been modified with permission from Chattergoon et al. 2013[34].

Table 1: Description of parameters used by the field-cycling relaxometer.

DISCUSSION:

The use of DNP to enhance signal acquisition is a technical solution to insufficient magnetic resonance signal available from ^{13}C nuclei at limited concentrations, as those used in animal injections, but presents other experimental challenges. Each relaxation measurement shown in **Figure 7** represents a measurement of a uniquely prepared sample because it cannot be repolarized after dissolution for remeasurement. This inevitably leads to experimental variability due to minor differences in sample preparation during weighing of the sample and dissolution media or variations in the dissolution process itself, such as incomplete extraction and thorough mixing of the sample with the dissolution media. This variability may be partially assessed by measuring the pH of each pyruvate solution after relaxometry. Regardless of careful weighing of stock pyruvate/radical mixture and dissolution medium before insertion in the DNP apparatus to better than a milligram, in our experiments the pHs ranged from 5.5 to 8.3. We have chosen to reject any T_1 data outside the pH range 7.6 to 8.0.

As mentioned above, the solid-state polarization level for each sample was at least 95%, which was obtained in about one hour. The liquid-state polarization was not estimated for each sample; however, periodic quality assurance of the DNP system, using the same sample preparation, resulted in liquid state polarization levels of about 15%.

During sample preparation, metal ion contamination may occur from contact between the dissolution medium and the DNP dissolution fluid path. This possibility required the addition of disodium ethylenediaminetetraacetic acid (EDTA) to sequester any of the metal ion contamination and preserve spin-lattice relaxation.

Comparing the shuttling method used in reference[28] and the fast field cycling presented in this protocol, we can say that the shuttling method is only possible when the shuttle time is small in comparison to the relaxation time; otherwise, the average magnetic fields experienced during the shuttling time may have a significant effect. With the fast field cycling relaxometer we used, the user is in complete control of the switching time, which can go as low as 3 ms. However, for hyperpolarized substrates, a slow switching time is required to keep adiabaticity and not to destroy the polarization of the sample during filed transitions. In our experience, for hyperpolarized ^{13}C-pyruvic acid, a switching time as low as 50 ms does maintain the polarization, but we observed more consistent results using a switching time of 100 or 200 ms. This small transition time from relaxation to acquisition and back to relaxation fields is negligible in comparison to the measured T_1 times and has no systematic effect on these measurements. We consider that further research is required to establish the boundaries of adiabaticity of different hyperpolarized substrates at different magnetic fields.

Another important difference between the two methods is the range of magnetic fields, which is 2 mT to 18.8 T for the shuttling method and 0.237 mT to 0.705 T for the field cycling relaxometer.

In this regard we can see the two methods as complementary to each other. However, for in vivo studies with hyperpolarized compounds, magnetic fields of up to 3 T are more common.

At field strengths of less than 1 mT, stray magnetic fields from surrounding objects were observed to have a systematic effect on our relaxation measurements. To eliminate these fields, we designed and added a custom magnetic shim around the field-cycling magnet. In comparison, the shuttling method uses μ-metal cylindrical shielding that produces an abrupt change of magnetic field from about 2 mT to 0.2 mT.

Temperature control of the sample was important due to the relatively long acquisition times requiring 300 to 510 s to capture the entire decay curve. We pre-warmed the NMR tubes prior to dispensing the hyperpolarized solution and then maintained the sample temperature by blowing warmed, temperature-regulated (37 °C) air over the tubes during relaxometry. This is an important advantage of the field-cycling relaxometer over the shuttling method because the temperature of the sample can be precisely controlled since the sample is stationary during measurements.

In addition, it was not practical to control the sample exposure to ambient temperature and magnetic field during the brief transfer time between polarizer and relaxometer. The T_1 of samples were measured at known magnetic fields and temperature controlled by the relaxometer, so transportation had limited influence. Conditions during transportation can only affect the amount of hyperpolarization that survives for measurement at the relaxometer. A portable holding field magnet (10 mT) was developed for transferring the hyperpolarized solution to the imaging magnet or relaxometer; however, its use was not worthwhile in this experiment given the brief transfer time but may be useful for other hyperpolarized liquids with greater T_1-dispersion at lower magnetic fields. A holding field of 0.01 T would increase the T_1 of the pyruvate solution by nearly 18% during transportation; however, with our relatively short transfer time of 8 s, these measurements suggest that only a 2.3% increase in signal would be observed.

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DISCLOSURES:

The authors have no disclosures.

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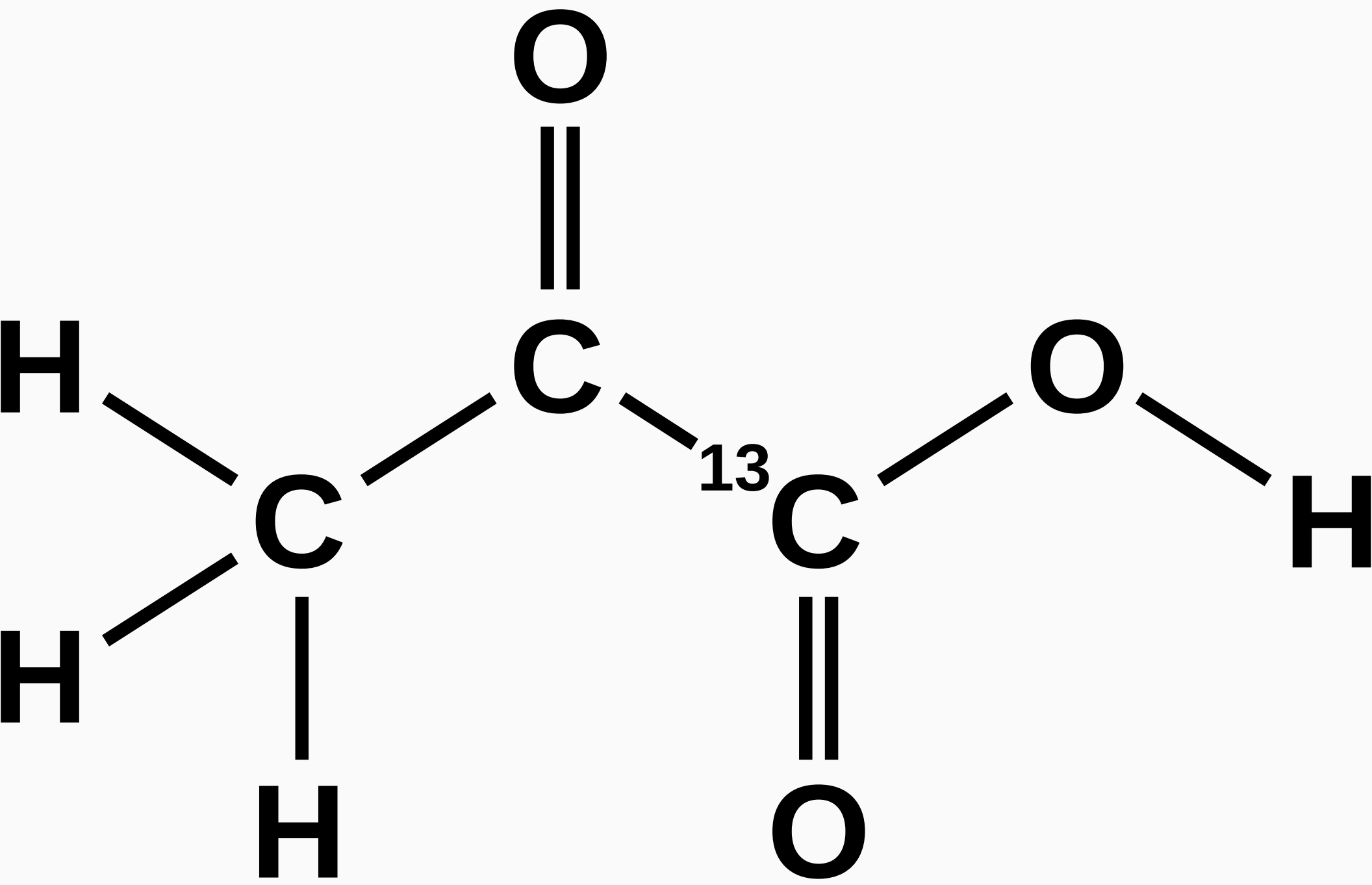
Figure



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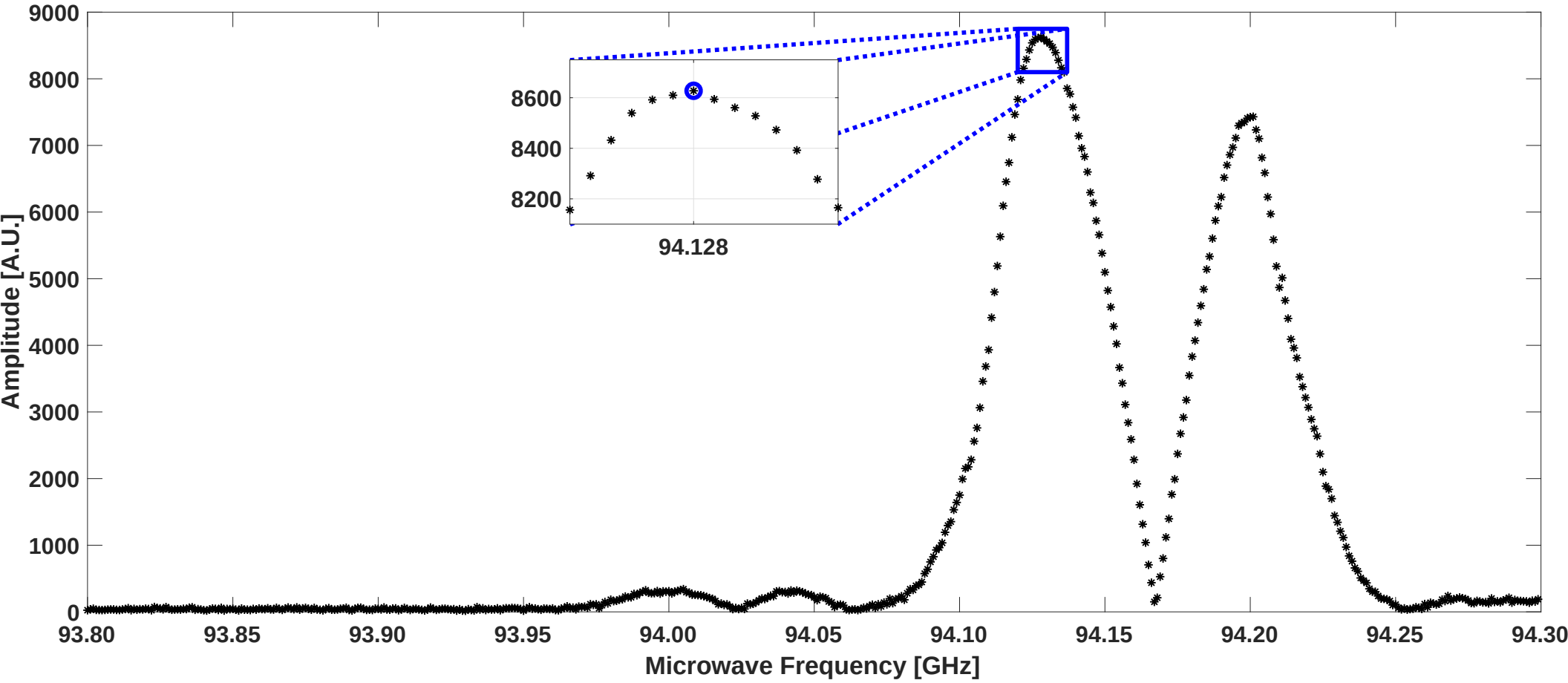

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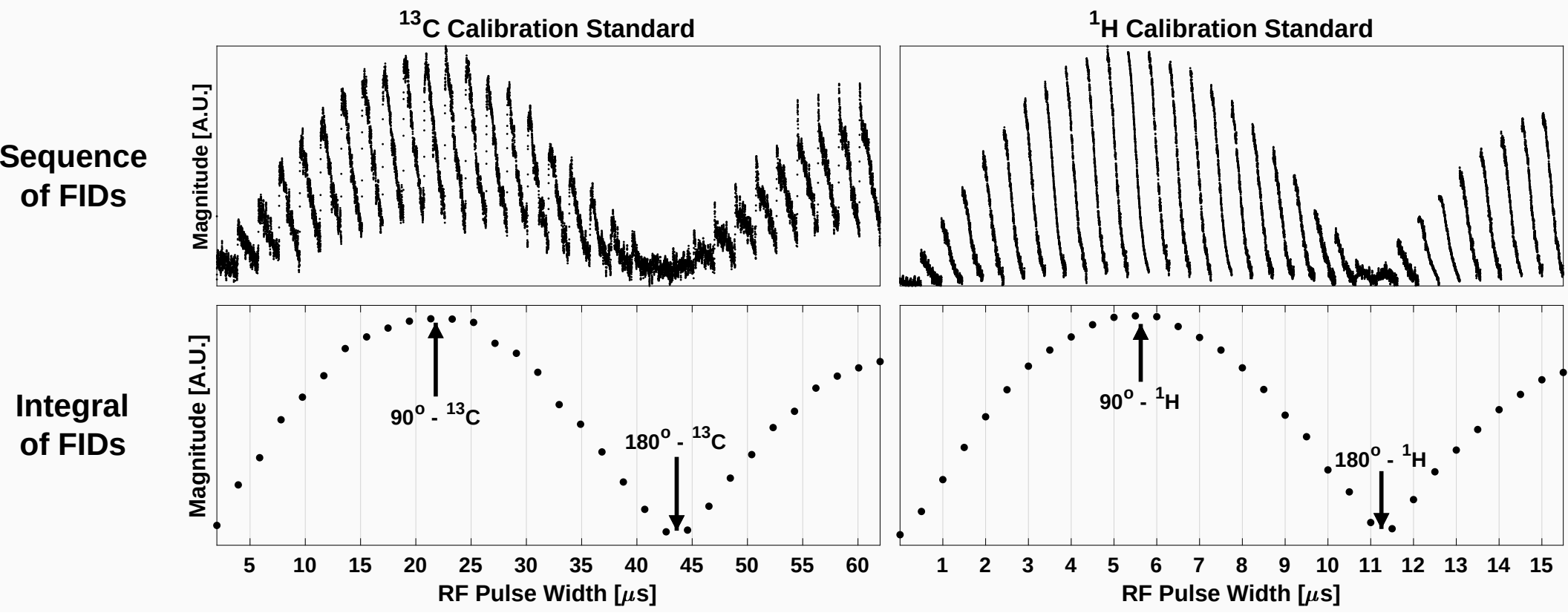

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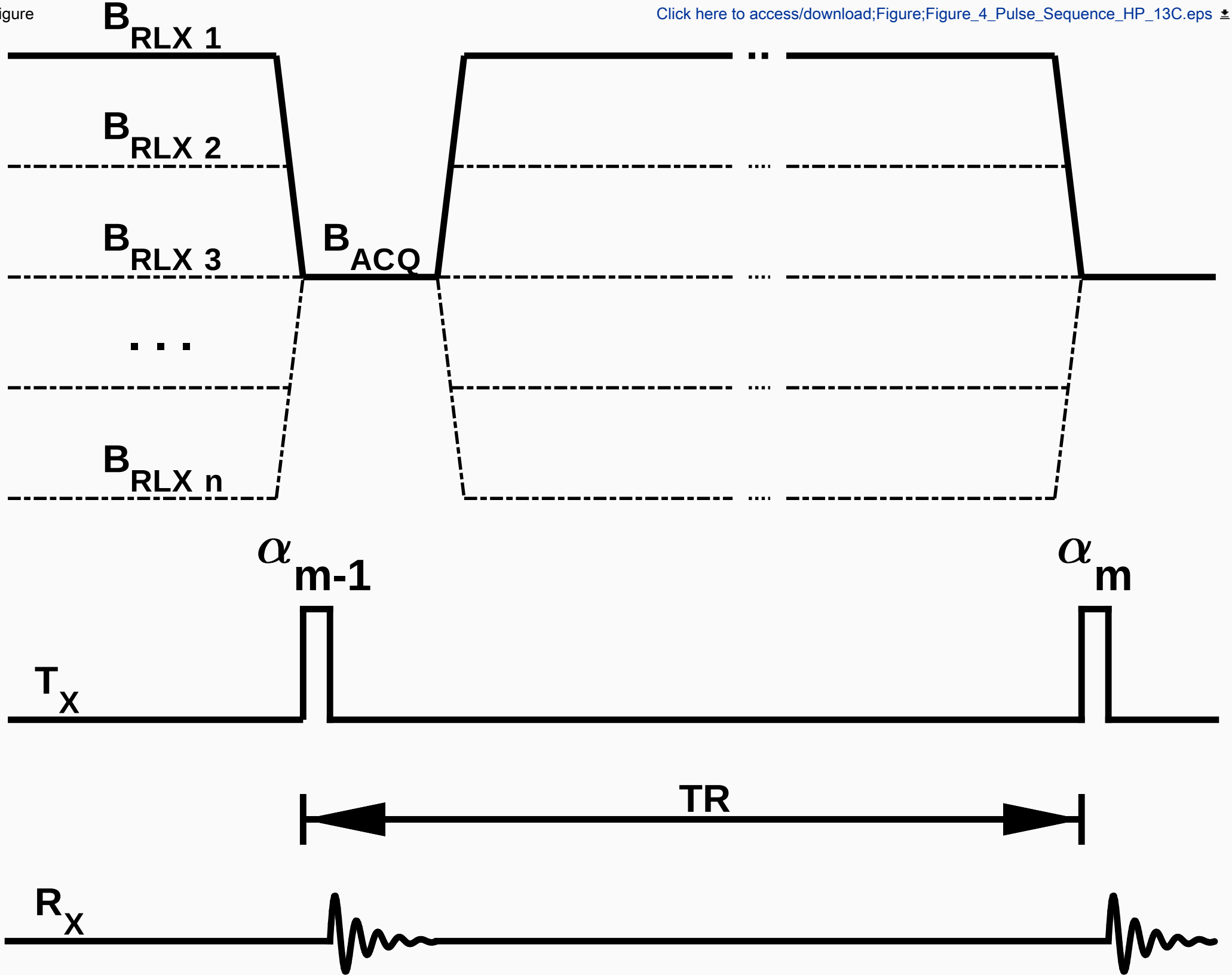

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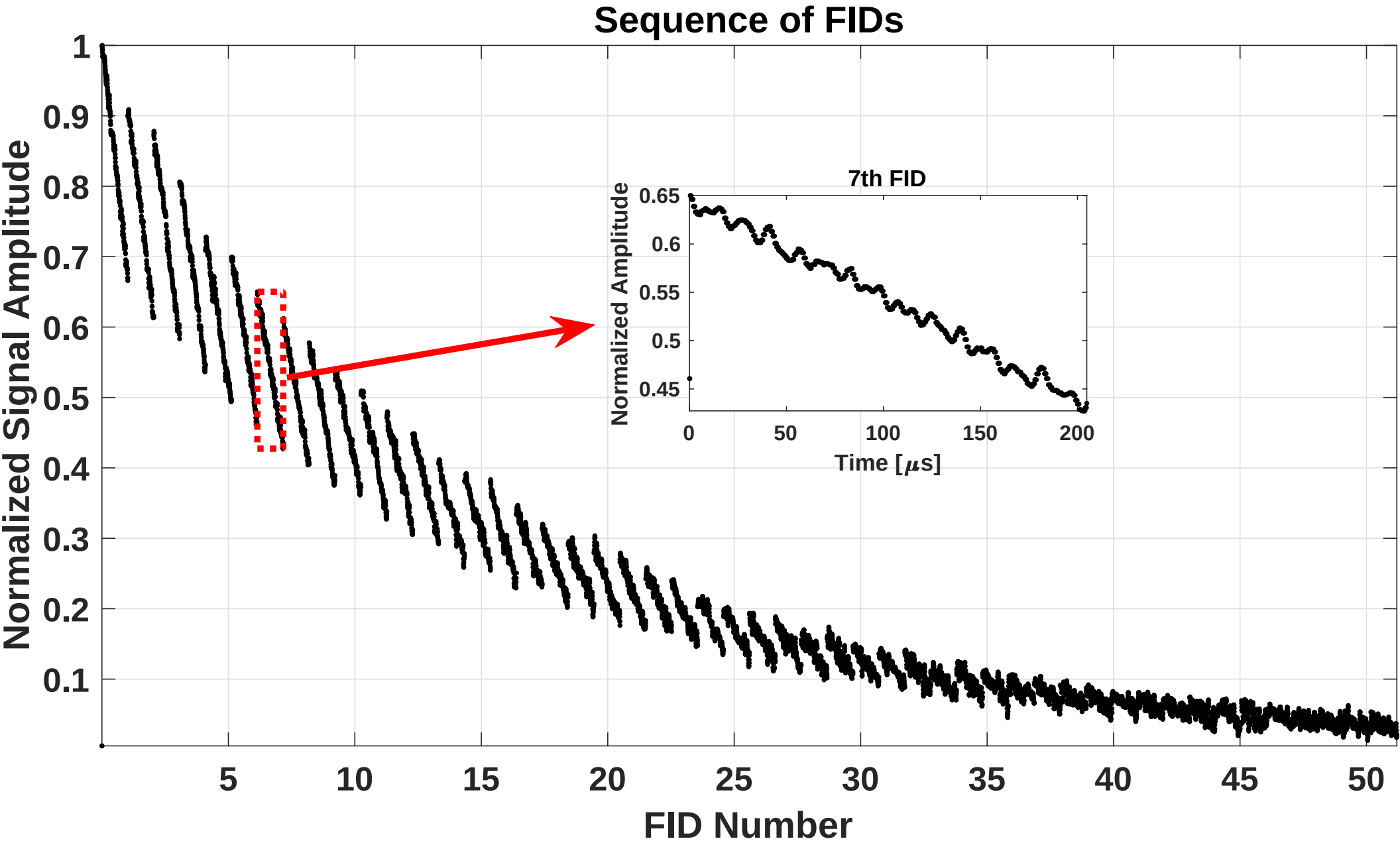

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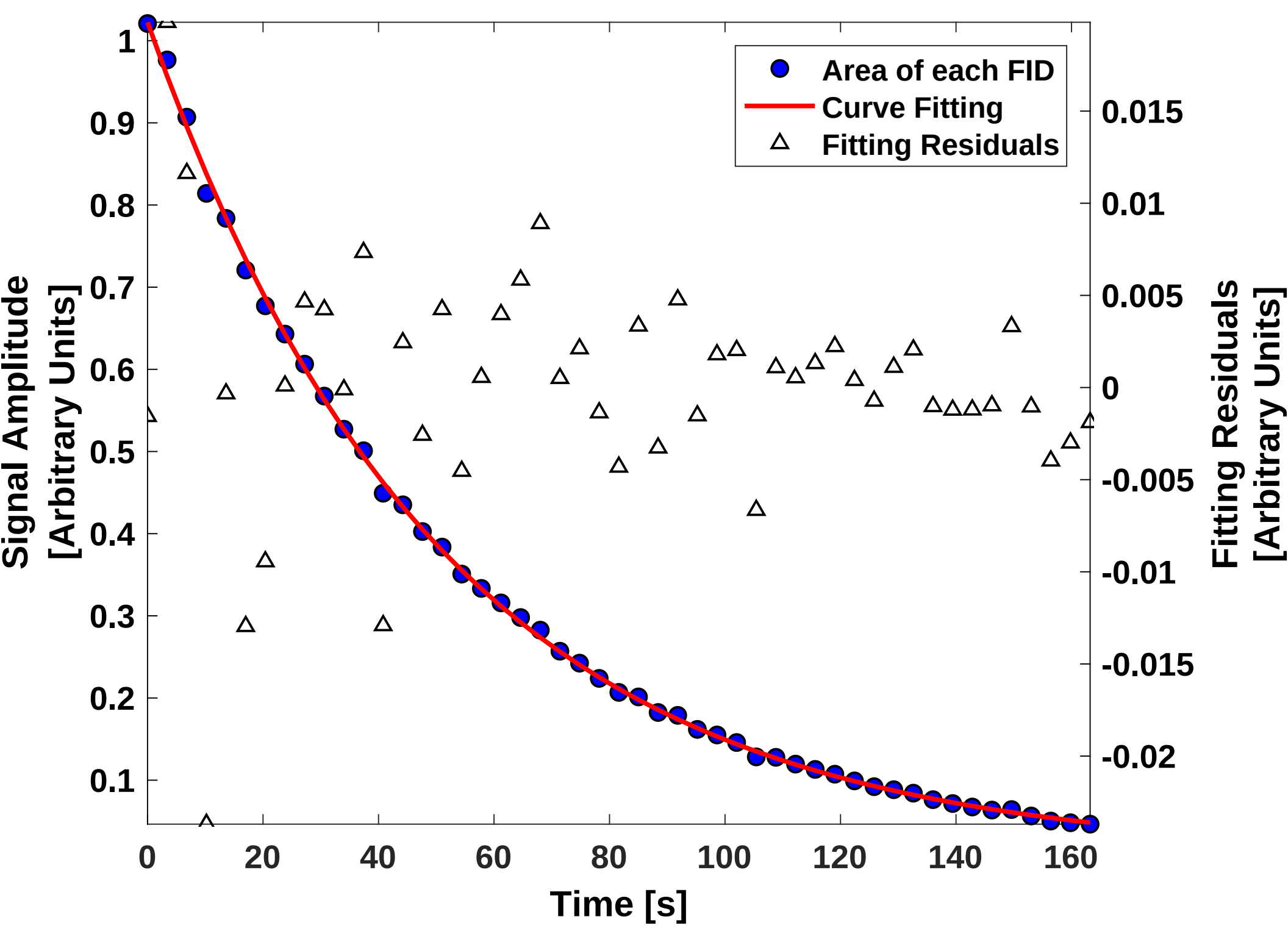

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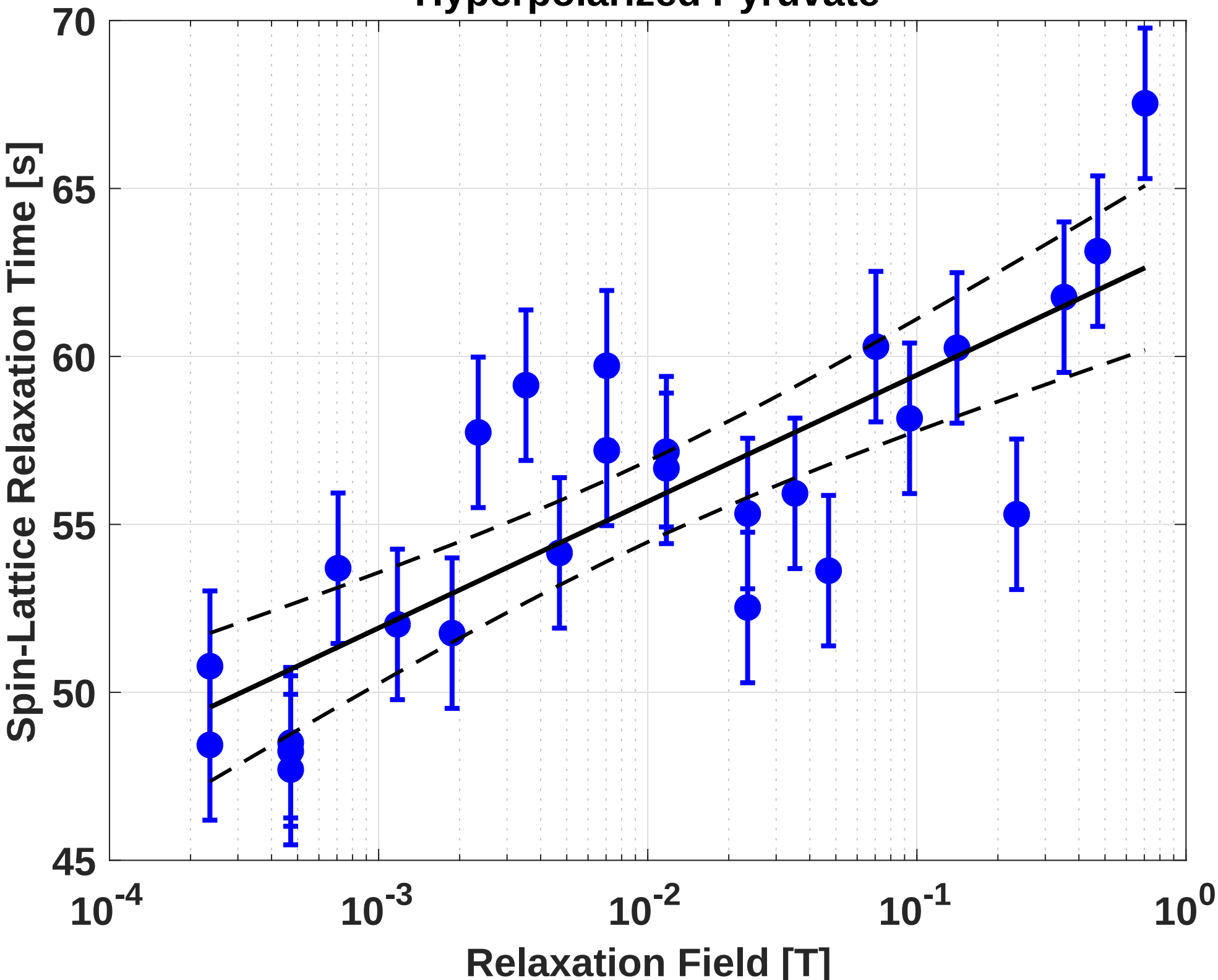

Table 1

Parameter	Short Description	Comments
ACQD	Acquisition delay	Delay required to allow magnetic field to reach steady state after transition and before data acquisition
BACQ	Acquisition Field	Specified by means of ^{1}H Larmor frequency
BEND	End value	Final value of the arrayed parameter
BINI	Initial value	First value of the arrayed parameter
BPOL	Polarization Field	Specified by means of ^{1}H Larmor frequency
BRLX	Relaxation Field	Specified by means of ^{1}H Larmor frequency
BS	Block size	Number of data points in a single block
EWEB	End block	Any integer number in the range of Number of Blocks (NBLK). 0 means "all"
EWEP	End point	Any integer number in the range of Block size (BS). 0 means "all"
EWIB	Initial block	From 1 to number of blocks (NBLK)
EWEP	Initial point	From 1 to block size (BS)
EXP	Experiment	Name of pulse sequence to be used
FLTR	Observe filter	Cutoff frequency of the audio signal filters
MS	Maximum scans	Desired number of averages
NBLK	Number of blocks	Number of sections for the arrayed parameter. The arrayed parameter is "PW90" for "13CANGLE" and "ANGLE" pulse sequences and "T1MX" for "HPUB/S" pulse sequence. PW90 changes after each repetition but T1MX remains constant.
NUC	Nucleus	For this protocol ^{13}C or ^{1}H
PW	Main RF pulse	Tip angle
PW90	90deg pulse	Duration of the 90-degrees pulse
RD	Recycle delay	Pre-scan magnet-cooling interval
RFA	RF attenuation	RF receiver attenuation
RINH	Receiver inhibit	Delay required to allow the decay of RF-coil ringing
SF	System Frequency	Larmor frequency used during acquisition
SW	Sweep Width	Spectral window width (Nyquist Frequency)
SWT	Switching time	Global magnet-switching time
T1MX	Maximum T1	Parameter used by the HPUB/S pulse sequence to define the polarization time during each repetition
TPOL	Polarization time	Parameter used by the "ANGLE" and "13CANGLE" pulse sequence to define the polarization time during each repetition

Units
µs
MHz
MHz
MHz
Hz
Degrees (°)
µs
s
dB
µs
MHz
Hz
s
s
s

Name of Material/Equipment	Company	Catalogue Number
[1-^{13}C]Pyruvic Acid	Sigma-Aldrich, St. Louis, MO, USA	677175
10mm NMR Tube	Norell, Inc., Morganton NC, USA	1001-8
De-ionized water		
Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA)	Sigma-Aldrich, St. Louis, MO, USA	E5134
HyperSense Dynamic Nuclear Polarizer	Oxford Instruments, Abingdon, UK	
MATLAB R2017b	MathWorks, Natick, MA	
OX063 Triarylmethyl radical	Oxford Instruments, Abingdon, UK	
pH meter - SympHony	VWR International, Mississauga, ON., Canada	SB70P
ProHance	Bracco Diagnostics Inc.	
Pure Ethanol (100% pure)	Commercial Alcohols, Toronto, ON, Canada	P016EAAN
Shim Coil		
Sodium Chloride	Sigma-Aldrich, St. Louis, MO, USA	S7653
Sodium Hydroxide	Sigma-Aldrich, St. Louis, MO, USA	S8045

SpinMaster FFC2000 1T C/DC	Stelar s.r.l., Mede (PV) Italy	
Trizma Pre-Set Crystals (pH 7.6)	Sigma-Aldrich, St. Louis, MO, USA	T7943

Comments/Description
Includes the following: "DNP-NMR Polarizer" software used to control and monitor the whole DNP polarizer; "RINMR" used to monitor the solid state polarization levels; "HyperTerminal" used to communicate the DNP software with the RINMR software that monitors the solid state polarization level. Also includes the MQC bench top spectrometer to monitor the liquid state polarization in conjunction with it own RINMR software
Include scripts for non-linear fitting of magnetization decay over time and T1 NMRD analysis of hyperpolarized pyruvic acid.
Gadoteridol, Gd-HP-DO3A
Developed in-house

Includes the software "AcqNMR" that is used to set experimental parameters, monitor the tuning and matching of the RF coil, loading different pulse sequences, calibrate flip angle, data acquisition and curve fitting, among other functions. Also includes a depth gauge, some weights and a depth stopper.

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Article Title: Measuring the spin-lattice relaxation magnetic field dependence of hyperpolarized [1-13C] pyruvate

Signature: Tim Scholl Date: Nov 13, 2018

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Response to Editorial Comments:

1. There are still some areas of overlap-please see the attached iThenticate report and substantially revise lines 46-49, 79-82, 109-111, 374-378, 397-400, 434-436, and 442-445.

The mentioned paragraphs have been edited.

2. With the revisions, the protocol is now over our limit for filming (2.75 pages; see attached). Please highlight 2.75 pages or less of the Protocol (including headers and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

The important steps to create a cohesive story are now highlighted.

3. It looks like Figures 6 and 7 are reused from a previous publication-please obtain explicit copyright permission to reuse them and any other figures that may have been reused. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

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4. There are a few badly-formatted symbols in the Results (the BINI and BEND parameters)-are those 'µ'?

Yes, those symbols should be 'µ'. They have been corrected and we hope they remain unchanged after this revision.

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