

Line-Narrowing by Polychromatic Selective Spin-Locking in NMR

Coline Wiame ¹, Hadi Loutfi ^{*,1}, Kirill Sheberstov ¹, Geoffrey Bodenhausen ¹

¹ Chimie Physique et Chimie du Vivant (CPCV, UMR 8228), Department of Chemistry,
5 Ecole Normale Supérieure, PSL University, Sorbonne University, CNRS, Paris, 75005,
France

**Corresponding author:* hadi.loutfi@ens.psl.eu

CW's ORCID: 0009-0008-3517-3354

HL's ORCID: 0009-0004-8617-6599

10 KS's ORCID: 0000-0002-3520-6258

GB's ORCID: 0000-0001-8633-6098

Abstract: Selective Spin-Locking (SSL) of the magnetisation vectors of selected singlets or multiplets in high-resolution Nuclear Magnetic Resonance (NMR) spectra by mono- or polychromatic selective radio-frequency (RF) irradiation can reduce the line-widths to the limit given by homogeneous $T_{1\rho}$ relaxation. The resulting line-narrowing leads to an increase in signal height of spectra of ^1H , ^{19}F , ^{31}P or other nuclei that is in principle only limited by the ratio $T_{1\rho}/T_2^*$. At the same time, the scalar interactions that give rise to multiplets are decoupled, thus leading to an additional gain in signal height. The amplification factors allow one to use samples at low concentrations. Common one-dimensional (1D) experiments such as inversion recovery (IR) as well as the excitation and reconversion of Long-Lived States (LLS) by Spin-Lock Induced Crossing (SLIC) can significantly benefit from SSL.

1. Introduction

Line-widths in NMR spectra are often broad due to inhomogeneous broadening arising from susceptibility effects, sample heterogeneity, or imperfect static field inhomogeneity. The SHARPER (Sensitive, Homogeneous, And Resolved PEaks in Real time) method enables exceptionally narrow NMR resonances by suppressing dephasing arising from chemical shift evolution and magnetic field inhomogeneities during acquisition (Jones et al., 2017; Davy et al., 2022; Dickson et al., 2022). In its conventional form, however, SHARPER can refocus only one or two resonance frequencies, precluding simultaneous linewidth reduction across multiple chemically distinct spins. To achieve line-narrowing on multiple peaks, we have developed a method that we shall refer to as Selective Spin-Locking (SSL), which is based on our earlier work on 'polychromatic decoupling' (Carnevale et al., 2012), where a number of short pulses with small flip angles are inserted in the intervals between the sampling points of the free induction decay (FID). Such short pulses are reminiscent of selective irradiation by Delays Alternating with Nutations for Tailored Excitation (DANTE) (Bodenhausen et al., 1976; Morris et al., 1978), and Irradiation of Narrow Frequency Envelopes by Repeated Nutation and Orbiting (INFERNO) (Morris et al., 1978), which are selective excitation methods that are widely used in NMR spectroscopy that use a comb of equidistant low-power RF pulses to achieve selective excitation of specific nuclear resonances. A suitable choice of the phases of these short pulses allows one to generate an irradiation spectrum where multiple peaks of interest are hit simultaneously. The combs of short pulses have the effect of *spin-locking* the magnetisation vectors belonging to the irradiated singlets or multiplets in their *local rotating frames*, thus preventing the phase dispersion due to the static field inhomogeneity and to *J-couplings*. In this case, the decay of the spin-locked transverse magnetisation will be determined by $\exp\{-t/T_{1\rho}\}$ rather than by $\exp\{-t/T_2^*\}$ so that the narrowest possible line-width, $\Delta\nu_{\min}^{\text{SSL}}$, is determined by the dissipation of the selectively spin-locked magnetisation in the rotating frame (Redfield, 1955), i.e., $\Delta\nu_{\min}^{\text{SSL}} = 1/\pi T_{1\rho}$.

Many common one-dimensional (1D) experiments designed to probe molecular motions can benefit from Selective Spin-Locking (SSL). Inversion Recovery (IR) experiments provide longitudinal relaxation times (T_1), enabling one to estimate rotational correlation times (τ_c), among other parameters. Carr-Purcell-Meiboom-Gill (CPMG) spin-echo experiments yield transverse relaxation times (T_2), allowing for the assessment of chemical exchange effects and other line-broadening mechanisms, such as scalar relaxation of the second kind. Other measurements that use pulsed field gradients (PFG) in order to determine diffusion coefficients can also benefit from SSL. Beyond 1D experiments, SSL can be combined with the many two-dimensional (2D) experiments used in NMR. For many studies, multiplet structures are essentially a nuisance, making the use of SSL valuable.

2. Mono- or Polychromatic Selective Spin-Locking (SSL)

60 The pulse sequence in Fig. 1 shows how mono- or polychromatic SSL during the acquisition of a Free Induction Decay (FID) is applied after a preparation period that can represent any arbitrary NMR experiment.

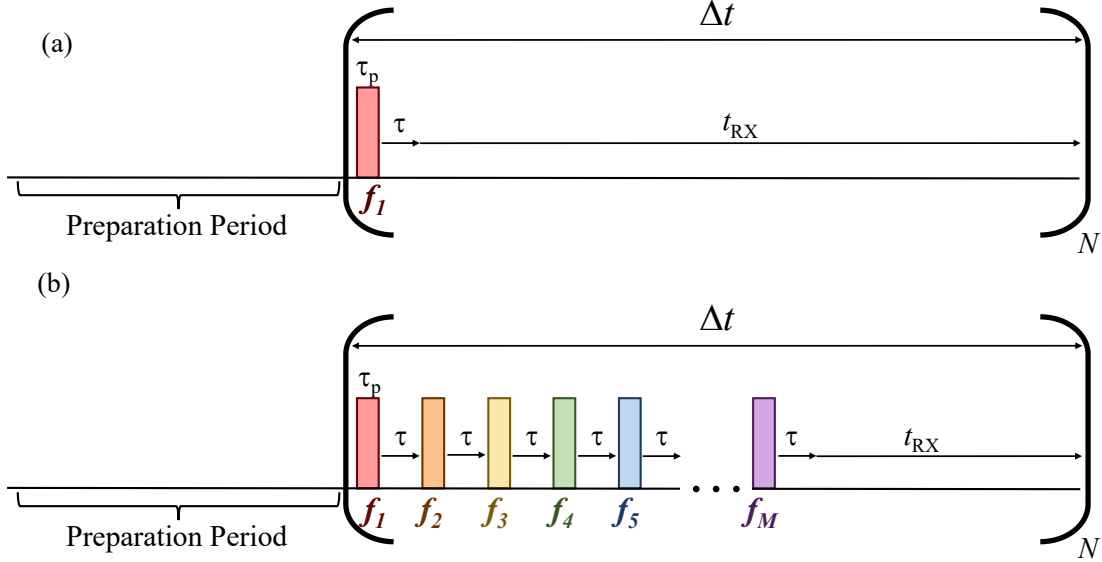


Figure 1: Schemes for (a) mono- or (b) polychromatic SSL, showing how a series of M short pulses separated by delays τ and associated with chemical shifts f_m (where $m = 1, 2, \dots, M$), each having a duration τ_p , can be applied during each interval Δt (typically equal to 150 μ s), to generate an FID. During the interval “ t_{RX} ” the receiver is activated to record sampling points, usually with oversampling. Each sequence of M short pulses is repeated N times, until the total number of points have been measured. Typically, $N = 32k = 32768$ in our experiments, so that the length of the FID is $N\Delta t = 4.92$ s. The preparation period may consist of a simple $\pi/2$ excitation pulse, a T_1 inversion recovery sequence, a T_2 CPMG echo sequence, or indeed one of the very large number of two-dimensional (2D) experiments where the SSL sequence can be used in the detection interval. For *in vivo* or *in vitro* Magnetic Resonance Spectroscopy (MRS), the sequence must be preceded by a method to select a volume of interest.

In SSL experiments, the preparation period usually includes a “hard” “non-selective” $\pi/2$ pulse, typically of 10 μ s for a peak RF amplitude $\omega_1/(2\pi) = 25$ kHz, followed by the SSL sequence comprising M short equidistant pulses applied during the acquisition block. The “soft” SSL pulses, separated by intervals $\tau = 1.1$ μ s, have a duration τ_p , which ranges between 1.5 to 7 μ s, with a peak RF amplitude on the order of 2 kHz. As a result, the individual pulses have small nutation angles in the range $1^\circ < \beta < 10^\circ$. Each sequence of M short pulses $\tau_p^{m,n}$ with $m = 1, 2 \dots M$ and $n = 1, 2 \dots N$ constitutes a “comb” in the manner of DANTE (Carnevale et al., 2012; Bodenhausen et al., 1976; Morris et al., 1978). The phase of the $\pi/2$ pulse must be cycled from x to -x, to make up for artefacts arising from RF breakthrough. The phases of SSL pulses are applied along y, so that they are shifted by 90° with respect to the $\pi/2$ pulse but remain parallel between SSL pulses. The phase of the receiver must be perpendicular

to the $\pi/2$ pulse, going from -y to y. These considerations entail that SSL experiments require a minimum of two scans.

To irradiate M chemical shifts f_m simultaneously, one must apply a superposition of pulses $m = 1, 2, \dots, M$, incrementing their phases from one interval Δt to the next ($n = 1, 2, \dots, N$) by phase-shifts $\Delta\Phi_m$, $n = n\Delta\phi_m$, where the “phase roll” $\Delta\phi_m$ can be calculated by the following equation:

$$\Delta\phi_m = 2\pi\Delta t\Delta f_m \quad (1)$$

where, as shown in Fig. 2, Δf_m corresponds to the difference in frequency (in Hz) between the RF carrier frequency and the m^{th} peak of interest. Using Δf_m , the phase shift required to apply the m^{th} short pulse, $\Delta\phi_m$, to the m^{th} peak of interest can be calculated using equation (1). For monochromatic SSL (Fig. 1a), the carrier frequency can be set to be on-resonance with the peak of interest so that $\Delta\phi_m = 0^\circ$.

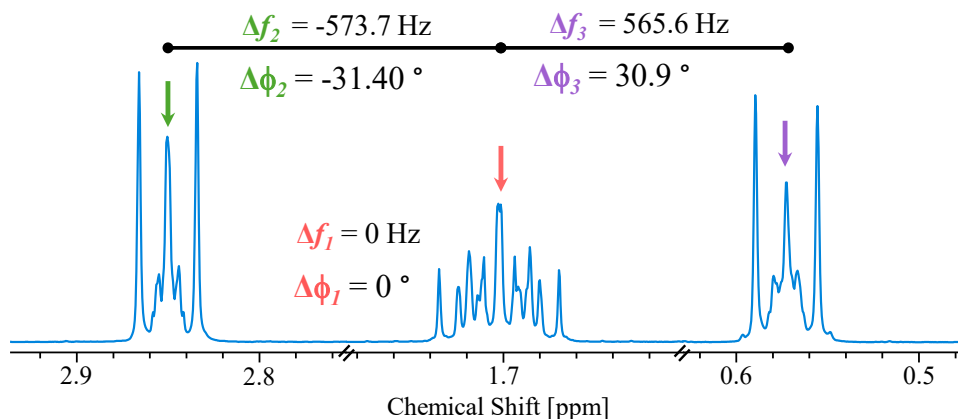


Figure 2: One-dimensional (1D) ^1H spectrum showing peaks corresponding to the three CH_2 groups of 200 mM sodium trimethylsilylpropanesulfonate (DSS) in 100 mM phosphate buffer solution (PBS, pH 7.2) in D_2O at 11.7 T (500 MHz for ^1H) showing the phase incrementation required for trichromatic SSL ($M = 3$) with $\Delta t = 152 \mu\text{s}$, the carrier frequency being set on the central peak (red arrow). The green and purple arrows designate the positions irradiated using phase incrementation which are calculated with Eq (1) to coincide with the central lines of the high- and low-frequency multiplets.

Signal acquisition occurs during a short time interval, designated by the delay “ t_{RX} ” in the sequences above, during which the receiver is activated in so-called oversampling mode. As illustrated in Fig. 1, the delay Δt remains the same for mono- or polychromatic sequences; the variable delay is “ t_{RX} ” which becomes shorter for experiments with large number of SSL pulses, M . The delay Δt is equal to two times the dwell time, to ensure the receiver has enough time to acquire the sampling points. This implicates that two data points, one real and one imaginary, are acquired in each SSL loop. The total number of points is observed after N repetitions of the SSL sequence (Fig. 1, bracketed section). Typically, N can be as large as 32 k or more. One can replace the intervals marked “ t_{RX} ” in Fig. 1 by

empty delays, in which case the line-narrowing method can be inserted in one of several evolution
 100 intervals of n -dimensional experiments.

The peak RF field amplitude of the short pulses is typically $\omega_1/(2\pi) = 2$ kHz, so that the average RF field strength, is proportional to the duty cycle:

$$\langle \omega_1/(2\pi) \rangle = \omega_1/(2\pi) \tau_p/\Delta t \quad (2)$$

In our work, the duty cycle is typically $\tau_p/\Delta t = 0.0125$ so that $\langle \omega_1/(2\pi) \rangle = 25$ Hz in our examples,
 105 corresponding to ca. 2 mW for each of the M interleaved DANTE sequences, well below the maximum power that can safely be applied to commercial solution-state NMR probes. The average RF field amplitude should be comparable to $1/(\pi T_2^*)$ if one wishes to narrow a single inhomogeneously broadened line, and comparable to J if one wishes to collapse a multiplet at the same time.

3. Monochromatic SSL for Line-Narrowing in the Absence of Couplings

110 The long-range J -couplings of the nine magnetically equivalent methyl protons in sodium trimethylsilylpropanesulfonate (DSS), to neighbouring protons are negligible, so that one can focus on the ability of SSL to overcome the effects of B_0 inhomogeneity. A conventional proton spectrum was measured after a $\pi/2$ pulse, using a 5 mm Bruker NEO iProbe at 11.7 T (500 MHz for ^1H), where a full width at half-height (FWHH) of approximately 1 Hz was achieved by rough shimming (Fig. 3, blue
 115 line). By deliberately mis-setting the z_1 -gradient of Bruker's room temperature shim system, the line was broadened to ca. 40 Hz (Fig. 3, light blue line).

As mentioned before, the smallest line-width that can be achieved is determined by the transverse relaxation time in the rotating frame, $T_{1\rho}$ (Redfield, 1955). For the methyl protons of DSS, $T_{1\rho} = 3.04$ s at 298 K and 11.7 T, as measured by applying the INFERNO method (Morris et al., 1978) with
 120 increasing durations $0.001 < \tau < 40$ s after an initial $\pi/2$ excitation pulse. Therefore, the theoretical minimum line-width that can be achieved in the case of these methyl protons is $\Delta\nu_{\min} = 1/\pi T_{1\rho} = 0.105$ Hz. The T_1 of these protons is equal to 3.13 s; the fact that $T_{1\rho}$ and T_1 are nearly equal shows that the extreme narrowing regime ($\omega_0\tau_c < 1$) is fulfilled to a good approximation.

The monochromatic SSL sequence (Fig. 1a), was applied to the middle of the inhomogeneously
 125 broadened resonance of the methyl groups of DSS, setting the duration of the short pulses $\tau_p = 2.85$ μs (nutaton frequency = 2.0 kHz for ^1H), applied within $\Delta t = 152$ μs (spectral width 6579 Hz), to achieve an average RF amplitude $\langle \omega_1/(2\pi) \rangle = 37.5$ Hz. The monochromatic SSL leads to a narrow peak (dark blue line in Fig. 3) with a $\Delta\nu = 0.23$ Hz, about 2.2 times broader than the theoretical limit $\Delta\nu_{\min} = 0.105$ Hz. This demonstrates that SSL with very weak RF fields can overcome severe inhomogeneous
 130 broadening. Monochromatic SSL was also applied to the well-shimmed proton signal (Fig. 3, blue line) with $\Delta\nu = 1.0$ Hz. The resulting line-narrowed spectrum, not shown in Fig. 3, also led to a $\Delta\nu = 0.23$

Hz. The line-narrowed peaks resulting from both the inhomogeneously broadened peak and from the well-shimmed peak show the same line-widths illustrating the ability of SSL to surmount different degrees of broadening.

- 135 The line-narrowing by a factor of ca. 5, leads to a gain in signal-to-noise ratio (SNR) by a factor of ca. 5. Note the slight displacement of the line-narrowed peak (Fig. 3, dark blue line), with respect to the conventional 1D spectrum peak arises from problems in accurately determining the centre of gravity of the inhomogeneously broadened light blue line. To verify that the quantitative aspect of proton NMR is conserved when applying monochromatic SSL, we compared the integrals of the conventional 1D and line-narrowed spectra and found them to be equal.

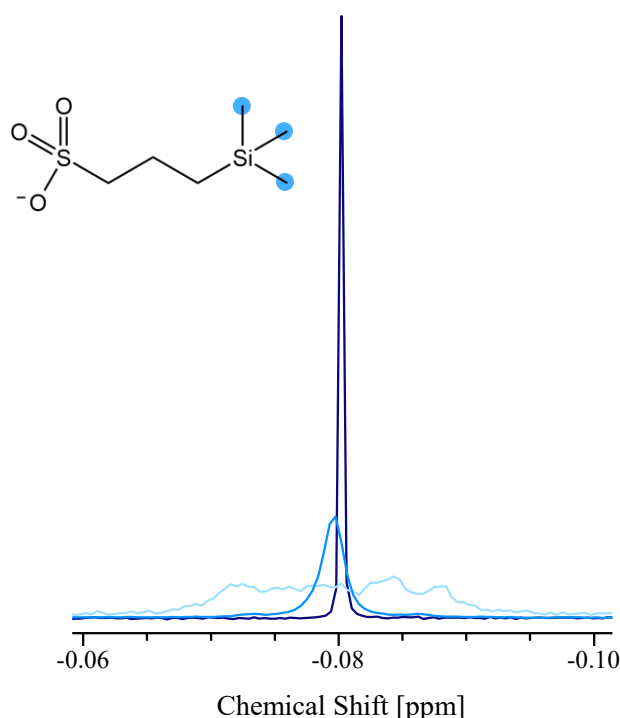


Figure 3: Line-narrowing by monochromatic SSL applied to the three equivalent CH₃ groups (blue circles) of 200 μM DSS in D₂O. Blue line: Conventional 1D spectrum with a line-width $\Delta\nu = 1$ Hz. Light blue line (scaled x2): Broadened to a line-width of about 10 Hz by deliberately miss-setting a shim current. Dark blue line: Line narrowed to $\Delta\nu = 0.23$ Hz by monochromatic SSL applied to the broadened light blue resonance, as described in the text. All spectra were obtained with 64 k points and 2 scans.

4. Monochromatic SSL for Line-Narrowing and Decoupling of Multiplets

4.1 Applications to Proton NMR

145 4.1.1 Combination with one-dimensional spectroscopy

In ¹H NMR spectra, methylene protons (–CH₂–) often appear as complex second-order multiplets due to couplings between magnetically inequivalent neighbouring CH₂ protons. Here, we measured a conventional well-shimmed spectrum excited by a $\pi/2$ pulse (Fig. 4, blue line), which

features a complex multiplet structure covering about 40 Hz and a central line with a $\Delta\nu = 2$ Hz. The
 150 inhomogeneously broadened peak (Fig. 4, light blue line) spans an overall width of about 50 Hz. The
 same monochromatic SSL experiment applied to the methyl groups of DSS, described in the previous
 section, was applied to the central CH_2 inhomogeneously broadened multiplet (Fig. 4, light blue line)
 of DSS giving rise to a narrow singlet (Fig. 4, dark blue line). A narrowing of the line-width, to
 $\Delta\nu = 0.27$ Hz, and a collapse of the multiplet is observed, boosting the SNR of the CH_2 peak in the SSL
 155 experiment by a factor of 22.

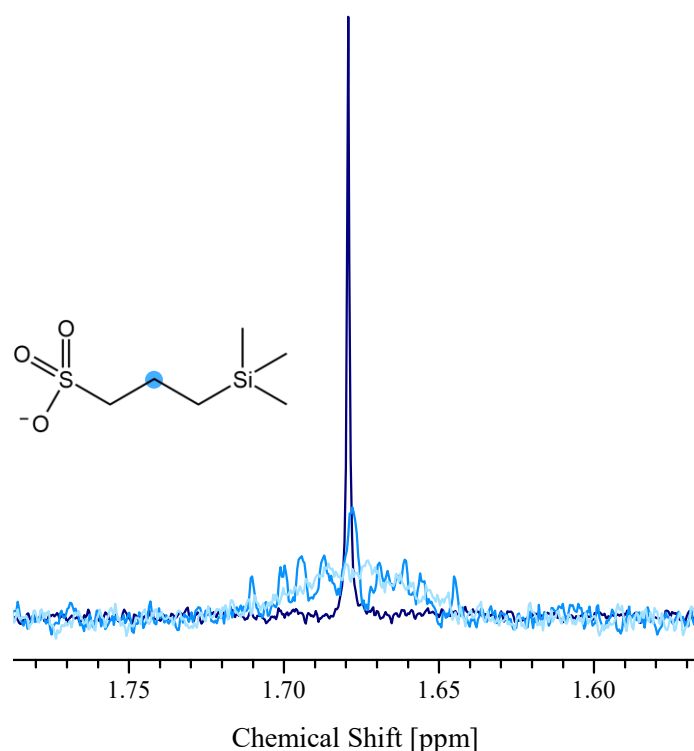


Figure 4: Line-narrowing and decoupling by monochromatic SSL applied to the central CH_2 multiplet (blue circle) of 200 μM DSS in D_2O . Blue line (scaled x2): Conventional 1D multiplet with an overall width of ca. 40 Hz and a $\Delta\nu = 2$ Hz for the central line. Light blue (scaled x2): Broadened by deliberately mis-setting a shim current to an overall width of ca. 50 Hz. Dark blue line: The broadened spectrum was line narrowed and decoupled to a line-width $\Delta\nu = 0.27$ Hz by monochromatic SSL, resulting from the Fourier transformation of an FID obtained with short pulses of $\tau_p = 2.85$ μs , applied within delays of $\Delta t = 152$ μs , with an average RF amplitude of 37.5 Hz. All spectra were obtained with 64 k points and 2 scans.

4.1.2 Combination with Long-Lived States (LLS)

In recent years, we have developed methods to excite long-lived population imbalances (Bocan
 et al., 2012) and reconvert them into observable magnetisation in $(\text{CH}_2)_n$ chains (Sonnenfeld et al., 2022a)
 160 by Spin-Lock Induced Crossing (SLIC) (Sonnenfeld et al., 2022b). The decays of the signals allow one
 to determine the lifetimes T_{LLS} of the superpositions of two-, four- or even six-spin order LLS terms.

Applying monochromatic SSL ($\tau_p = 2.85 \mu\text{s}$, applied within $\Delta t = 152 \mu\text{s}$, and 37 Hz average RF amplitude) to a multiplet that results from the reconversion of LLS in the low-frequency CH_2 group of β -alanine (Fig. 5b) collapses the multiplet of an overall width equal to 18 Hz and narrows the central line-width of 1 Hz to a sharp singlet of $\Delta\nu = 0.3 \text{ Hz}$. The SNR of the peaks is increased by factors close to 3 (Fig. 5) after achieving monochromatic SSL, where it's particularly welcome for these types of experiments. Indeed, at least 90 % of the signal is lost during the creation and reconversion of LLS by SLIC experiments, so that boosting the peak height by SSL offers a significant advantage. The LLS lifetime was extracted by mono-exponential fitting and was found to be $T_{\text{LLS}} = 17 \text{ s}$, for experiments with and without SSL.

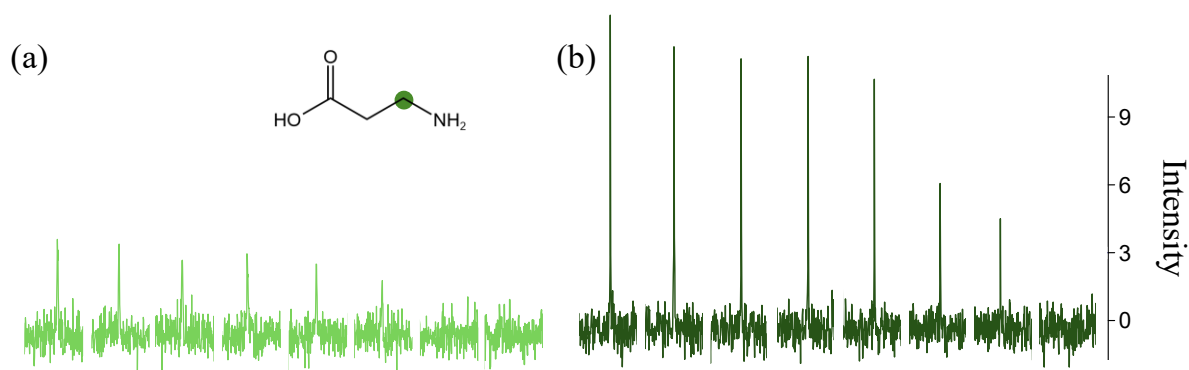


Figure 5: LLS signals of the low-frequency CH_2 group (green circle) of 2 mM β -alanine in 10 mM PBS (pH 7.2) in D_2O , obtained by the reconversion of LLS into magnetisation by SLIC. The LLS were created on both CH_2 groups by monochromatic SLIC-in and reconverted into magnetisation by monochromatic SLIC-out. (a) Without SSL one observes a $\Delta\nu = 1 \text{ Hz}$. (b) With monochromatic SSL, as described in the text, applied to the low-frequency CH_2 group during acquisition, the line-width is narrowed to $\Delta\nu = 0.3 \text{ Hz}$. The interval between the SLIC-in and SLIC-out pulses was incremented in 8 steps of increasing size going from 0.1 to 30 s. The SNR is increased by a factor of ca. 3 when SSL is applied. Both experiments were obtained with 64 k points and 2 scans.

4.2 Applications to Fluorine-19 NMR

4.2.1 Combination with one-dimensional spectroscopy

Fluorine-19 spectra tend to show complicated multiplets because of the ubiquity of long-range couplings. Fig. 6 shows an application of a *monochromatic* SSL sequence (at 11.7 T or 470.53 MHz for ^{19}F and 298 K) to perfluorobutanesulfonic acid (PFBS), a typical example of per- and poly-fluoroalkyl substances (PFAS). SSL experiments were measured on two samples of PFBS with concentrations (a) 20 mM and (b) 200 μM shown in Fig. 6. The monochromatic SSL sequence was applied, for both samples, to the central CF_2 group of PFBS, with short pulses of $\tau_p = 3.8 \mu\text{s}$ (nutation frequency = 2.1 kHz for ^{19}F) applied within delays $\Delta t = 21.2 \mu\text{s}$ (spectral width 47.17 kHz) for an average RF amplitude of 120 Hz. These parameters were optimised to effectively collapse the entire multiplet which covers a region of ca. 76 Hz. This experiment results in a line-narrowing effect decreasing the FWHH from about 22 to 0.45 Hz for Fig. 6a, while the SNR is increased by a factor of

ca. 26. For the 200 μM PFBS sample (Fig. 6b), the FWHH decreases from 22 Hz to 0.65 Hz, while the
 185 SNR is increased by a factor of ca. 11.

In molecules that are not perfluorinated but contain residual protons our method allows one to dispense with proton decoupling since both homo- and heteronuclear couplings are effectively suppressed.

The transverse relaxation time in the rotating frame of these ^{19}F nuclei was measured by the INFERNO method (Morris et al., 1978) to be $T_{1\rho} = 1.39$ s, so that the narrowest theoretically possible line-width
 190 achievable by SSL for these nuclei is $\Delta\nu_{\text{min}} = 0.23$ Hz. The experimental value measured is therefore ca. 2 times larger, for Fig. 6a and ca. 3 times larger for Fig. 6b than this limit.

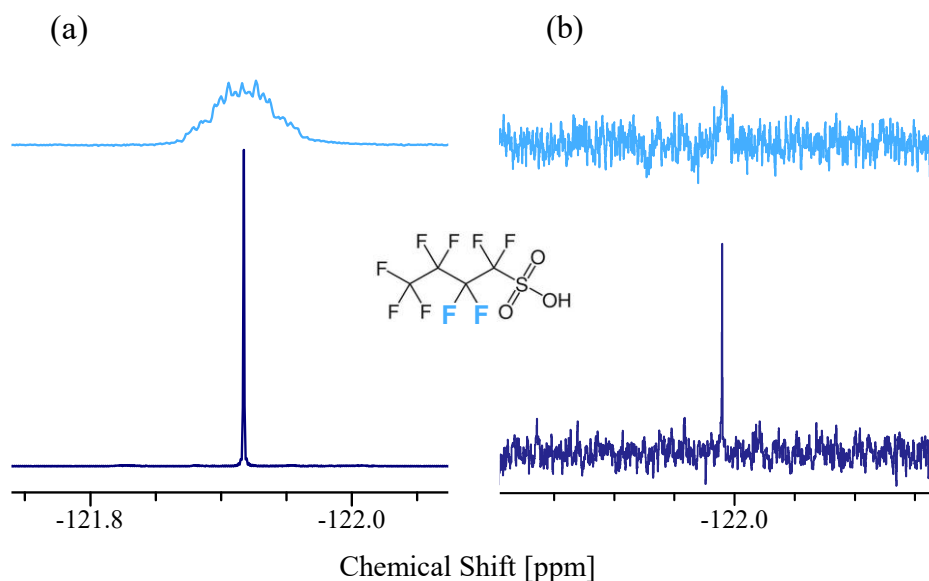


Figure 6: Fluorine-19 spectra of the central CF_2 multiplet (fluorine atoms highlighted in blue) of (a) 20 mM and (b) 200 μM PFBS in D_2O . Light blue line: Conventional 1D spectrum for (a), scaled x4, having an overall width of about 76 Hz and a FWHH of 22 Hz and for (b) an overall width of about also 76 Hz and FWHH of 22 Hz. Dark blue line: Line narrowed and decoupled to a line-width $\Delta\nu = 0.45$ Hz for (a) and for $\Delta\nu = 2$ Hz (b) by monochromatic SSL as described in the text. All spectra were obtained with 64 k points and 2 scans.

4.2.2 Combination with Long-Lived States (LLS)

In recent work, we investigated the creation and reconversion of LLS in $(\text{CF}_2)_n$ chains using
 195 SLIC (Wiame et al., 2025). The excitation yield of LLS in these fluorinated aliphatic chains by SLIC is only around 1 %, making SSL even more appealing than for applications to protons. Fig. 7 shows the signal decay of LLS without and with monochromatic SSL ($\tau_p = 3.8$ μs , applied in delays $\Delta t = 42.0$ μs for an average RF amplitude of 61 Hz) irradiating the central CF_2 peak of PFBS, resulting from the reconversion of LLS. Here, the LLS signal is line narrowed and decoupled, from a line-width of 20 Hz
 200 and an overall width of ca. 57 Hz to only $\Delta\nu = 0.5$ Hz, while increasing the SNR by a factor ca. 20. In both experiments, it was found that $T_{\text{LLS}} = 3.5$ s.

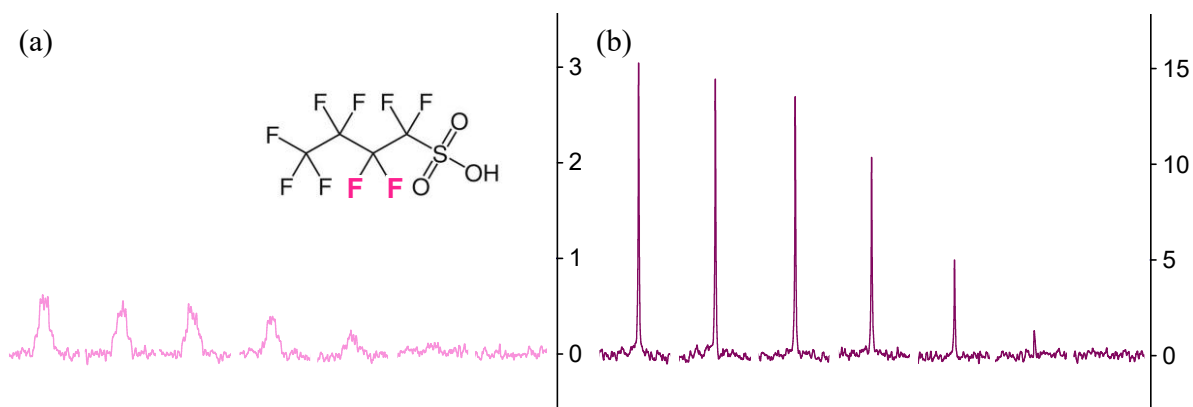


Figure 7: LLS signals of the central CF₂ group (highlighted in pink) of 20 mM PFBS in D₂O, obtained by the reconversion of LLS into magnetisation by SLIC. The LLS was created and reconverted by monochromatic SLIC-in and monochromatic SLIC-out. (a) Without SSL with overall span of about 57 Hz and a FWHH of 20 Hz. (b) With monochromatic SSL applied to the central CF₂ group during acquisition as described in the text, narrowing the line-width to $\Delta\nu = 0.5$ Hz. The interval between the SLIC-in and SLIC-out pulses was incremented in 8 steps of increasing size from 0.1 to 60 s. The intensities of all signals were increased by a factor of ca. 20. Both experiments were obtained with 64 k points and 2 scans. To increase the number of data points across each line, a Lorentzian line broadening of 0.3 Hz was applied so that each peak is represented by 9 points.

5. Polychromatic SSL for Line-Narrowing and Decoupling

5.1 Applications to Proton NMR: Combination with one-dimensional spectroscopy

205 In addition, a *dichromatic* SSL sequence ($M = 2$ in Fig. 1b) was applied to both CH₂ groups in the antibiotic metronidazole (MDZ), achieving narrowing and decoupling of both multiplets from a line-width of $\Delta\nu = 3$ Hz for the central line and an overall width of ca. 15 Hz of the multiplet to a singlet with a line-width of only 0.23 Hz (Fig. 8). The SNR of the two peaks are increased by factors close to 38 and 31 respectively. The parameters of the dichromatic SSL are $\tau_p = 1.9$ μ s (for a nutation frequency
210 of 1.9 kHz), applied within delays $\Delta t = 152$ μ s, with an average RF amplitude of 25 Hz. The carrier frequency was set half-way between the two multiplets (wavy arrow in Fig. 8) so that the effective irradiation frequencies appear at $\Delta f_1 = -150.8$ Hz and $\Delta f_2 = +150.8$ Hz with respect to the carrier.

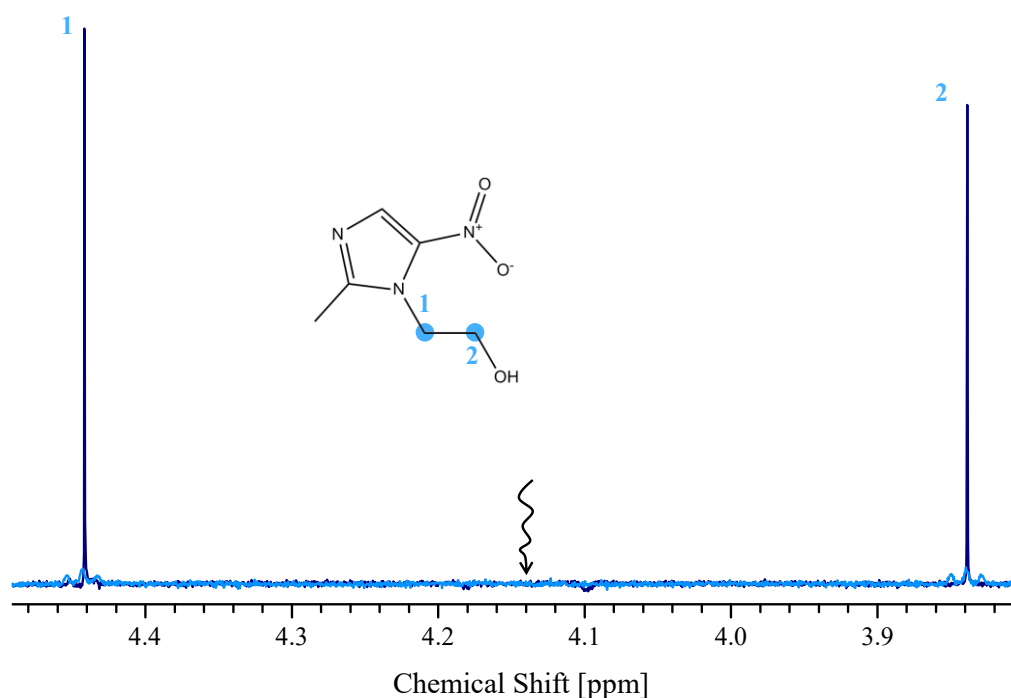


Figure 8: Line-narrowing and decoupling by dichromatic SSL applied simultaneously to the high- and low-frequency CH₂ peaks (highlighted by blue circles) of 200 μ M MDZ in D₂O at 11.7 T (500 MHz for ¹H) and 298 K. Blue line: Conventional 1D spectrum with a line-width $\Delta\nu = 3$ Hz. Dark blue line: Lines narrowed and decoupled to a line-width $\Delta\nu = 0.23$ Hz by dichromatic SSL as described in the text. The wavy arrow indicates the RF carrier frequency. The SNR of the two peaks are increased by factors close to 38 and 31 respectively. Both spectra were recorded with 64 k points and 2 scans.

When dichromatic spin-locking is applied to the chemical shifts A and X of a coupled two-spin AX
 215 system, the effects of all couplings J_{AM} , J_{XM} to non-irradiated nuclei are suppressed, but the coupling J_{AX} between the two irradiated spins may not be perfectly cancelled. This effect is reminiscent of “recoupling” in solid-state experiments (Tošner et al., 2021). Furthermore, one may observe undesirable coherence transfer phenomena because of homonuclear cross-polarization if the Hartmann-Hahn condition is fulfilled (Sonnenfeld et al., 2022b; Wiame et al., 2025). Such effects can be suppressed by
 220 using unequal average RF amplitudes of the selective fields applied to the chemical shifts A and X.

We applied an *octa-chromatic* SSL ($M = 8$ in Fig. 1b) sequence to a mixture of various small molecules, i.e., metronidazole, vitamin B1, acetylcholine, β -alanine, taurine and γ -aminobutyric acid (GABA), as shown in Fig. 9. In this experiment, we could effectively narrow and decouple overlapping peaks, even
 225 SSL were decreased by a factor of ca. 2, while the SNR are increased by factors ranging from 20 to 27. The same τ_p , average RF amplitude, and Δt used for the dichromatic SSL experiment described previously were used for octa-chromatic SSL. The carrier frequency was set to be on-resonance with the peak highlighted in light green, corresponding to a CH₂ group of acetylcholine (black wavy arrow in Fig. 9b) and the effective irradiation frequencies were $\Delta f_1 = 0$ Hz, $\Delta f_2 = +156.27$ Hz, $\Delta f_3 = +238.87$

230 Hz, $\Delta f_4 = +273.48$ Hz, $\Delta f_5 = +363.34$ Hz, $\Delta f_6 = -69.21$ Hz, $\Delta f_7 = -90.34$ Hz, $\Delta f_8 = -387.04$ Hz with respect to the carrier. The spectrum in Fig. 9b features a number of sharp spikes that do not arise from the nuclei but are due to intermodulation effects.

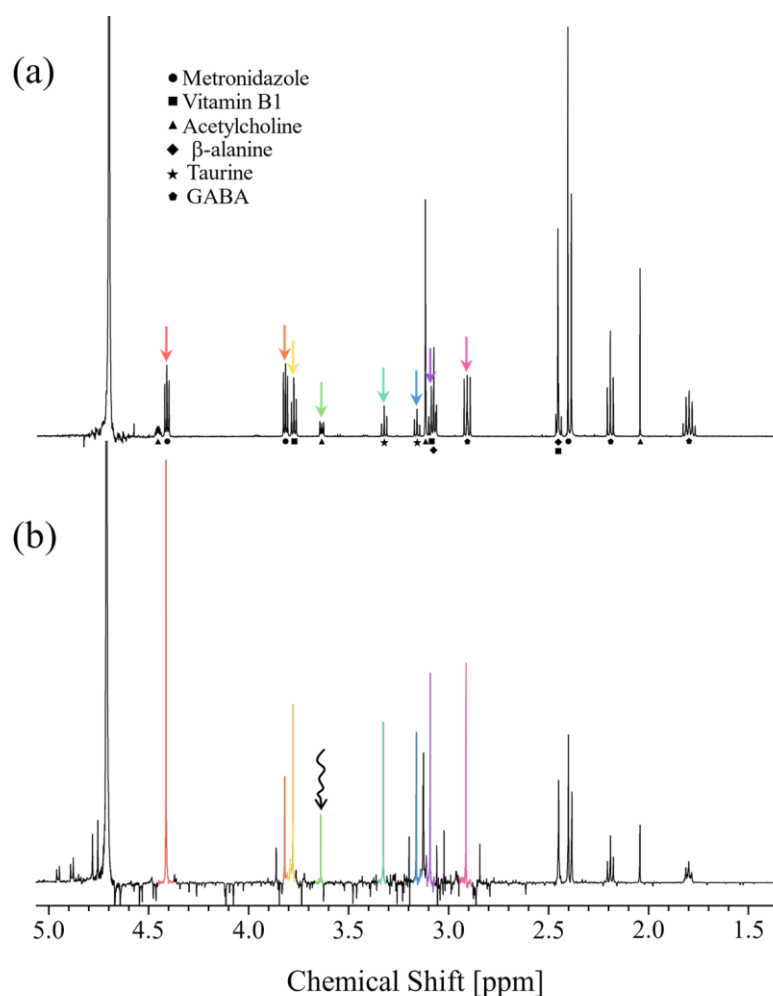


Figure 9: Line-narrowing and decoupling by octa-chromatic SSL applied to a mixture of six small molecules: metronidazole, vitamin B1, acetylcholine, β -alanine, taurine and GABA, all at 10 mM concentrations in 100 mM PBS (pH 7.2) in D_2O . (a) Conventional 1D spectrum. (b) Lines narrowed and decoupled spectrum obtained by octa-chromatic SSL as described in the text. The 8 coloured arrows in (a) correspond to the irradiation frequencies of the pulses chosen to spin-lock 8 selected proton signals. The colour-coded peaks in (b) show the corresponding irradiated peaks after line-narrowing and decoupling by octa-chromatic SSL. The wavy arrow indicates the RF carrier frequency. To increase the number of data point across the peaks, a Lorentzian line broadening of 0.3 Hz was applied so that each peak is represented by 5 points. The SNR are boosted by factors between 20 and 27. Both spectra were recorded with 16 k points and 2 scans.

5.2 Proton NMR: Combination with Inversion Recovery (IR)

235 We were also able to combine *dichromatic* SSL with the Inversion Recovery (T_1) experiment, as shown in Fig. 10b for the amino acid, β -alanine. The line-widths of the peaks equal to ca. 1 Hz and the overall width of the peaks equal to ca. 23 Hz are reduced to $\Delta\nu = 0.25$ Hz by dichromatic SSL and the SNR are increased by 11 and 8 for orange spectra and the blue spectra respectively. The T_1 was

extracted by mono-exponential fitting and found to be ca. 2.7 s, both without and with SSL. Adding an
 240 SSL block after an IR sequence therefore provides a robust method for measuring T_1 values while
 boosting the peak heights by a sizable factor.

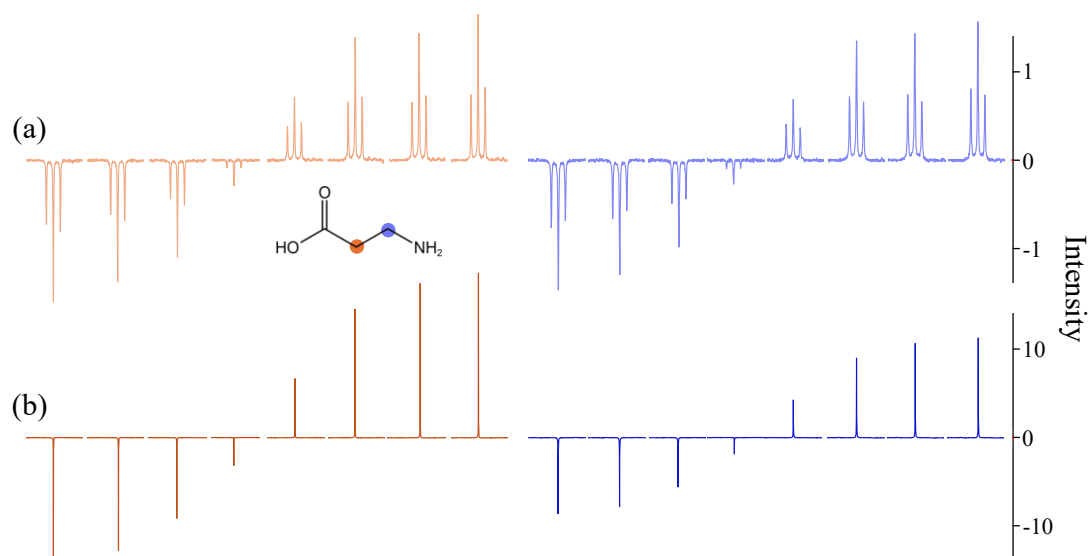


Figure 10: Inversion-Recovery (T_1) experiments showing the high-frequency CH₂ (orange) and low-frequency CH₂ (blue) groups of 2 mM β-alanine in 10 mM PBS (pH 7.2) in D₂O. (a) Without SSL with a line-width $\Delta\nu = 0.8$ Hz. (b) Lines narrowed and decoupled to line-widths close to $\Delta\nu = 0.24$ Hz by dichromatic SSL as described in the text. The SNR are increased by 11 and 8 for orange spectra and the blue spectra, respectively. The interval between the inversion pulse and the excitation pulse was incremented in 8 non-linear steps going from 0.1 to 30 s. Both experiments were obtained with 64 k points and 2 scans.

Likewise, T_2 CPMG spin-echo experiments can benefit from SSL. Such experiments (not shown in this
 245 work) were run and yielded accurate relaxation times T_2 .

6. Applications to Phosphorus-31 NMR

Although phosphorus-31 resonances at high fields are broadened due to rapid relaxation driven by the chemical shift anisotropy, which cannot be eliminated by SSL, various other line-broadening mechanisms are amenable to line-narrowing. To assess this, we looked at the three ^{31}P nuclei in
 250 Adenosine Triphosphate (ATP). In addition to the decoupling of homonuclear $J(^{31}\text{P}, ^{31}\text{P})$ interactions, a significant line-narrowing effect was observed (Fig. 11). In the conventional spectrum, the individual lines of the left-hand doublet have widths of about $\Delta\nu = 1.3$ Hz, those of the central doublet have $\Delta\nu = 13$ Hz, and lines of the right-hand triplet have $\Delta\nu = 2.3$ Hz. The two doublets span widths of about 20 Hz while the triplet spans a width of ca. 40 Hz. By applying *trichromatic* SSL to the all three chemical
 255 shifts, the multiplets are decoupled and their lines are narrowed to line-widths of 0.7 Hz, multiplying

the SNR by factors of ca. 0.9, 5.5, and 1.5 for the signals from left to right. One can notice a significant enhancement of the SNR for the P_α compared to the other signals which arises from the decoupling of hetero-nuclear coupling with neighbouring protons which broaden this doublet. Note that the noise level is increased in the SSL experiments of Fig. 11 by a factor of ca. 2. The optimised parameters for this

260 experiment were $\tau_p = 3.8 \mu\text{s}$, $\Delta t = 152 \mu\text{s}$ and $\langle\omega_1/(2\pi)\rangle = 35 \text{ Hz}$. The carrier frequency was set half-way between the outer multiplets at -16.9 ppm (black wavy arrow in Fig. 11). The irradiation frequencies were $\Delta f_1 = -1239.9 \text{ Hz}$, $\Delta f_2 = -1132.3 \text{ Hz}$ and $\Delta f_3 = +1239.9 \text{ Hz}$. The fact that the three peaks have nearly the same heights suggests that SSL yields quantitative intensities, i.e., that the peak heights are proportional to the numbers of spins and hence to the concentrations.

265 The transverse relaxation time in the rotating frame of the central ^{31}P nucleus was measured by the INFERNO method (Morris et al., 1978) to be $T_{1\rho} = 0.357 \text{ s}$, so that the narrowest theoretically possible line-width achievable by SSL for these nuclei is $\Delta\nu_{\min} = 1/\pi T_{1\rho} = 0.89 \text{ Hz}$. Surprisingly, the experimental line-widths were even narrower since a linewidth $\Delta\nu = 0.7 \text{ Hz}$ is measured at all three sites.

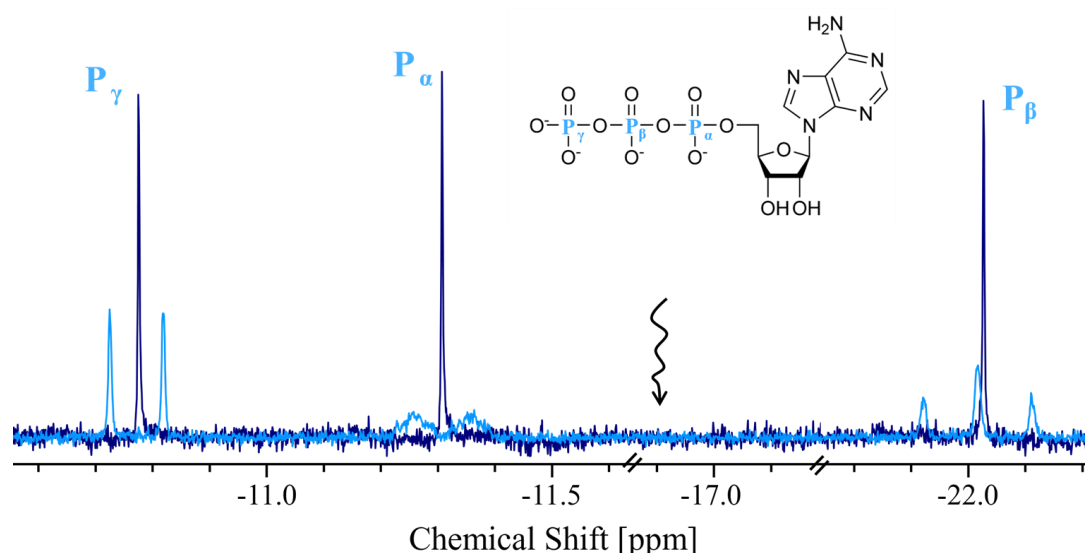


Figure 11: Phosphorus-31 spectra of 20 mM ATP (phosphorus atoms highlighted in blue) in D_2O at 11.7 T (202.41 MHz for ^{31}P) and 298 K. Blue line: Conventional ^{31}P spectrum. Black line: Line-narrowed and decoupled spectrum obtained by trichromatic SSL, as described in the text, with line-widths $\Delta\nu = 0.7 \text{ Hz}$ at all three sites. The wavy arrow indicates the RF carrier frequency. Both spectra were obtained with 64 k points and 2 scans.

7. Conclusion

270 By applying mono- or polychromatic SSL to selected singlets or multiplets in high-resolution NMR spectra of ^1H , ^{19}F , ^{31}P and other nuclei, one can collapse both homo- and heteronuclear scalar couplings, eliminate inhomogeneous broadening, and thus obtain very narrow signals. The decay of the spin-locked transverse magnetisation is in principle limited by $\exp\{-t/T_{1\rho}\}$ rather than by $\exp\{-t/T_2^*\}$, so that

the line-widths upon Fourier transformation can approach the theoretical limit $\Delta\nu_{\min} = 1/\pi T_{1\rho}$ rather than
275 inhomogeneous width $1/\pi T_2^*$.

The reduction of line-widths and decoupling of multiplets leads to an increase in peak heights, ranging from factors 4 to 40 in the examples discussed in this work. This should make it possible to reduce the detection level, i.e., the minimum concentration of molecules that is required to detect their NMR signals, provided the identity of the molecules under investigation and their chemical shifts are known.
280 This may be of interest for metabolomic studies.

The SSL method should also be useful for special probes where several samples can be studied in parallel, where the static homogeneity and hence the line-widths may suffer from imperfect shimming (Van Dyck et al., 2026).

Applications of SSL are not limited to small molecules. The line-widths in proton spectra of
285 macromolecules at high fields are usually dominated by homogeneous T_2 relaxation, and $T_{1\rho}$ is usually close to T_2 rather than T_1 as in small molecules, so that line-narrowing by SSL is not likely to be very effective. However, although decoupling of homo- and heteronuclear scalar interactions (Cavanagh et al., 2007) such as $J(^1\text{H}, ^1\text{H})$, $J(^{13}\text{C}, ^1\text{H})$ and $J(^{15}\text{N}, ^1\text{H})$ may be useful in macromolecules. Furthermore, the lines can be broadened by chemical exchange, in particular by exchange between major and minor
290 conformers, and the effects of SSL should be investigated empirically.

All magnetic resonance imaging (MRI) methods achieve spatial resolution by using pulsed field gradients, including “frequency encoding” during signal observation. Spin-locking should obviously not be applied during signal observation (Mosher et al., 1990). However, in a sub-class of MRI experiments that have come to be known as magnetic resonance spectroscopy (MRS), where the signals
295 are observed in the absence of gradients to determine the chemical shifts, spin-locking can be applied during signal observation. This can be used inter alia to determine the concentrations of various constituents or metabolites in the brains of rodents and humans, as well as phosphorylated molecules like ATP (Chung et al., 2024), phosphocreatine, etc., in various organs. At high fields, the line-widths in phosphorus-31 spectra are usually dominated by homogeneous T_2 relaxation, particularly if the
300 chemical shift anisotropy (CSA) is the dominant line-broadening mechanism. A priori, such line-widths are not amenable to narrowing by SSL. However, we have shown that significant line-narrowing can be achieved in addition to decoupling of $J(^{31}\text{P}, ^{31}\text{P})$ interactions. Furthermore, the line-widths of ^1H , ^{31}P or ^{13}C resonances in vivo are broadened by discontinuities in tissues, variations in susceptibility, and motions due to arterial or respiratory effects. Such static or dynamic line-broadening effects should be
305 largely mitigated by mono- or polychromatic SSL during signal acquisition.

In Magic Angle Spinning (MAS) solid-state NMR, the line-widths in proton spectra are usually dominated by residual dipole-dipole interactions (Chávez et al., 2021) that cannot be suppressed by SSL.

However, the line-widths in MAS spectra of ^{13}C and other dilute nuclei are often broadened by disorder in amorphous or polycrystalline materials. Their line-widths could therefore be narrowed by SSL, in as far as the modulations due to sample spinning do not interfere with the short pulses used in SSL. Synchronisation of these pulses with the rotor period can alleviate such problems (Caravatti et al., 1983).

In solids spinning at the magic angle, half-integer spins like ^7Li and ^{23}Na feature central transitions between the $m = \pm 1/2$ levels that are broadened by inhomogeneous second-order quadrupole interactions. Such line-shapes could be collapsed to their average positions by applying SSL near the frequency of their first moment. A superposition of M overlapping sites (as occurs in battery materials) could be separated by a two-dimensional method where M -chromatic SSL is applied in the detection period but not in the evolution interval.

The SSL method might be applicable to other two-level systems such as occur in optical spectroscopy, although broadening by Doppler effects might not be amenable to line-narrowing.

A wide range of methods collectively known as “pure shift spectroscopy” have been derived from two-dimensional J-spectroscopy (Aguilar et al., 2010; Castañar et al., 2015). These ingenious methods allow one to collapse multiplets due to scalar couplings. While the final result bears similarities to mono- or polychromatic SSL, in particular since decoupling is accompanied by line-narrowing (in principle limited only by the homogeneous relaxation rate $1/\pi T_2$ for pure shift spectroscopy), the mechanisms are quite distinct, and mono- or poly-chromatic spin-locking is more efficient in terms of signal-to-noise per unit time. Similar considerations can be applied to the SHARPER method (Jones et al., 2017; Davy et al., 2022; Dickson et al., 2022). By contrast, the DREAMTIME method (Jenne et al., 2022) uses doubly selective irradiation tailored for known compounds.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

Authors’ contributions

KS and GB designed the experiment. CW and HL carried them out. CW, HL, KS and GB prepared the manuscript.

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are built on the same principles as SSL but focused on decoupling and tickling without taking advantage
340 of line-narrowing.

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KeyWords: Spin-locking • Relaxation in the rotating frame • Polychromatic decoupling•
Inhomogeneous line-broadening.

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