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HORRENDOUS NMR: Establishing correlations in solution-state NMR by reinstating non-secular J-coupling terms

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ABSTRACT

Homonuclear isotropic mixing modules allow J-coupled spins to exchange magnetization even when separated by chemical shift offsets that exceed their couplings. This is exploited in TOtal Correlation SpectroscopY (TOCSY) experiments and its variants, which facilitate these homonuclear polarization exchanges by applying broadband RF pulses. These then establish an effective Hamiltonian in which chemical shift offsets are erased, while J-coupling terms –including flip-flop components– remain active. The polarization that these non-secular terms will transfer among systems of chemically inequivalent sites over the course of a mixing period, are widely used modules in 1D and in multidimensional liquid-state NMR. Homonuclear correlation experiments are also common in solids NMR, particularly among X = ^{13}C or ^{15}N nuclei. Solids NMR experiments are often challenged by high-power RF demands which have led to a family of homonuclear solid-state correlation experiments that avoid pulsing on the nuclei of interest, and focus instead on the ^{1}Hs that are bonded to them. These solid experiments usually reintroduce/strengthen ^{1}H-X dipolar couplings; these, in conjunction with assistance from rotational resonance effects, bring back the truncated X-X dipolar interactions and facilitate the generation of cross peaks. The present study explores whether a similar goal can be achieved for solution-state counterparts, based on the reintroduction of truncated flip-flop terms in the J-coupling Hamiltonian via the pulsing on other, heteronuclear species. A proposal to achieve this is derived, and the resulting HOmonucleaR Recoupling by hEteroNuclear DecOUplingS (HORRENDOUS) approach to provide correlations between like nuclei without pulsing on them, is demonstrated.

1. Introduction

Homonuclear TOtal Correlation SpectroscopY (TOCSY) is a well-established technique finding extensive use in solution-state NMR [1–4]. TOCSY facilitates magnetization transfers within mutually coupled spin networks by applying RF pulse sequences that erase chemical shift differences among the spins, allowing otherwise truncated, non-secular J-coupling terms, to distribute polarization over the course of a mixing period [5–8]. TOCSY experiments are most widely used in ^{1}H NMR, where the abundance of these species, the relatively narrow range of chemical shift offsets, and the complexity of the spectra, make such 2D correlations straightforward and useful. The advent of uniform labeling schemes, the well-resolved nature of ^{13}C spectra, and the relatively large strength (>30 Hz) of ^{13}C–^{13}C ^{1}J-couplings, also enabled ^{13}C–^{13}C TOCSY experiments to become an important part of structural elucidations in biomolecular NMR [9,10]. These experiments tend to be more challenging than their ^{1}H-based counterparts, due to the high power requirements that TOCSY mixing sequences require to effectively spin lock carbons over the wider spectral range over which they resonate. This leads, particularly at high fields and/or in lossy samples, to sample heating problems [11–13]. Similar complications can arise in solid-state NMR, where homonuclear correlations among ^{13}C also play an important role in enabling biomolecular studies [14–18]. Non-secular homonuclear ^{13}C–^{13}C couplings in solids, however, involve a major dipolar component, meaning that their effects will be influenced by both truncation effects and by a magic-angle spinning (MAS) which is not used in solutions [19,20]. As a result of these multiple features, numerous ^{13}C–^{13}C correlation experiments have been proposed where homonuclear interactions in spinning solids are reintroduced by manipulating the ^{1}Hs that are present in the system; i.e., not the species whose correlations are being sought, but rather heteronuclear species that couple strongly to them. These include methods

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such as DARR, MIRROR, RAD, and AL-FRESCO, where ^{1}Hs manipulations are timed with the MAS, so as to reinstate the effects of truncated ^{13}C–^{13}C (or ^{15}N–^{15}N) couplings [20–27]. While these experiments rely on dipolar couplings that are not meaningful in isotropic liquids, solution-phase counterparts might also exist where isotropic J couplings drive similar effects. This study explores one such approach, which in the tradition of "daunting" acronyms in solids NMR recoupling, we have termed HOmonucleaR Recoupling by hEteroNuclear DecOUplingS (HORRENDOUS). The HORRENDOUS idea for recoupling homonuclear pairs by manipulating heteronuclear couplings is derived in the next paragraph, and experimentally tested in the following sections.

2. Theoretical considerations

We consider for simplicity an H-C$_1$-C$_2$ system of three bonded spins, which is the simplest I-S$_1$-S$_2$ ensemble where homonuclear (S$_1$-S$_2$) correlations can be imparted by manipulating a third (I) spin. Further, we assume that in this I-S$_1$-S$_2$ system J-couplings are only active among directly bonded spins (i.e., among species connected by the "-" hyphen), and that the two carbons are separated by an offset $\Delta\Omega \gg J_{S_1S_2}$ that truncates the $J_{S_1S_2}$ flip-flop terms between S$_1$ and S$_2$. HORRENDOUS's idea to unquench the ensuing truncation involves the application of a nutation field ω_{1I} on the I-spin that will introduce a modulation in the effective J_{IS_1} coupling. One way of visualizing how this could partially reintroduce the non-secular $J_{S_1S_2}$ terms is by considering the S$_1$ decoupling sidebands that will be introduced by a continuous wave (CW) I-spin irradiation [28–30]: when these overlap with the S$_2$ spectral line, one can expect non-secular contributions to be reactivated. In an interaction representation defined by the I, S$_1$ and S$_2$ chemical shifts [31,32], this can be understood on the basis of time-dependencies arising on the homonuclear flip-flop terms due to the $\Delta\Omega$-imposed evolution affecting its zero-quantum sub-space, $\frac{J_{S_1S_2}}{2}\left(S_{1+}S_{2-}e^{-i\Delta\Omega t} + S_{1-}S_{2+}e^{i\Delta\Omega t}\right)$, as well as on the heteronuclear coupling term $J_{IS_1}\left[\{I_x\cos(\omega_{1I}t) + I_y\sin(\omega_{1I}t)\}\right]S_{1z}$ arising as result of the ω_{1I} irradiation. When these two time-dependencies are equal a non-zero, time-independent term will arise in the second-order average Hamiltonian that ensues from these two non-commuting terms, bringing back the action of the $S_{1\pm}S_{2\mp}$ flip-flops, and thereby enabling S1 ↔ S2 transfers.

To consider a more general case we assume that the CW I-decoupling is not necessarily on resonance, but rather applied at an offset Ω_I along the x axis. The Hamiltonian that describes all interactions in the three-spin system is then

$$H = -\Omega_I I_z - \Omega_{1S}S_{1z} - \Omega_{2S}S_{2z} + 2\pi J_{IS_1}I_zS_{1z} + 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{1}{2}\left(S_{1+}S_{2-} + S_{1-}S_{2+}\right)\right] - \omega_{1I}I_x \tag{1}$$

where $\Omega_I = \omega_{I,0} - \omega_{I,rf}$ and $\Omega_{iS} = \omega^i_{S,0} - \omega_{S,rf}$ (i = 1, or 2) are the offset frequencies of the I- and S-spins, respectively; $J_{\lambda_1\lambda_2}$, where λ s are I, S$_1$ or S$_2$ are the heteronuclear and homonuclear J-couplings; $S_{i\xi}$(i = 1, 2; ξ = z, +, or -) represent the S-spin longitudinal or transverse spin operators for the two spins; and $(S_{1+}S_{2-} + S_{1-}S_{2+})$ is the non-secular homonuclear J-coupling interaction term which HORRENDOUS is trying to reinstate. Considering the I-spin evolution in a tilted reference frame precessing at an effective frequency $\omega_{eI} = \sqrt{\omega_{1I}^2 + \Omega_I^2}$, Eq. (1) can be rewritten as:

$$\begin{aligned} H^{'} = & -\omega_{eI}I^{'}_z - \Omega_{1S}S_{1z} - \Omega_{2S}S_{2z} \\ & + 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{1}{2}\left(S_{1+}S_{2-} + S_{1-}S_{2+}\right)\right] \\ & + 2\pi J_{IS_1}\left[\cos\theta_I I^{'}_z + \sin\theta_I I^{'}_x\right]S_{1z} \end{aligned} \tag{2}$$

where $\cos\theta_I = \frac{\Omega_I}{\omega_{eI}}$, $\sin\theta_I = \frac{\omega_{1I}}{\omega_{eI}}$ define the tilting angle θ_I. This Hamiltonian imposes two implicit time-dependencies: one at frequency ω_{eI} driving the I-spin nutation, and another at $\Delta\Omega = \Omega_{1S} - \Omega_{2S}$ driving the zero-quantum space evolution where the S-spin polarization transfer takes place. To account for these two, we transform the Hamiltonian in Eq. (2) into a doubly interacting frame, defined by commuting transformations $U_1 = e^{i\omega_{eI}tI_z}$ and $U_2 = e^{i(\Omega_{1S}S_{1z} + \Omega_{2S}S_{2z})t}$:

$$H_{int} = U_2U_1H^{'}U_1^{-1}U_2^{-1} + iU_2U_1\frac{d}{dt}\left(U_1^{-1}U_2^{-1}\right) \tag{3}$$

Dropping for simplicity the apostrophe from the I-spin operators (i.e., $I_i = I^{'}_i, i = x,\ y,\ \text{or}\ z$):

$$\begin{aligned} H_{int} &= H^{S_1,S_2}_{int}(t) + H^{I,S_1}_{int}(t) \\ &= 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{1}{2}\left(S_{1+}S_{2-}e^{-i\Delta\Omega t} + S_{1-}S_{2+}e^{i\Delta\Omega t}\right)\right] \\ &\quad + 2\pi J_{IS_1}\left[\frac{\Omega_I}{\omega_{eI}}I_z + \frac{\omega_{1I}}{\omega_{eI}}\left\{I_x\cos(\omega_{eI}t) + I_y\sin(\omega_{eI}t)\right\}\right]S_{1z} \end{aligned} \tag{4}$$

The aforementioned interference effects imposed on the S$_1$-S$_2$ homonuclear coupling due to the I-S$_1$ heteronuclear J-coupling modulation, can be visualized by rewriting $H^{S_1,S_2}_{int}(t)$ in a third interaction frame specified by:

$$\tilde{H}{}^{S1,S2}_{int} = U_{I,S1}H^{S1,S2}_{int}(t)U^{-1}_{I,S1} + iU_{I,S1}\frac{d}{dt}U^{-1}_{I,S1} \tag{5}$$

where we approximate $U_{I,S1} = \exp\left\{i\frac{1}{t}\int_0^t \overline{H}^{IS_1}_{int}(t')dt'\right\} = \exp\{i\Xi(t)S_{1z}\}$. By assuming $\langle I_i\rangle = Trace(I_i\sigma_i) = 1$ where i = x, y, or z, and σ_i s are the Pauli matrices, one obtains that

$$\Xi(t) = 2\pi J_{IS_1}\left[\frac{\Omega_I}{\omega_{eI}}t + \frac{\omega_{1I}}{\omega_{eI}^2}\{\sin(\omega_{eI}t) - \cos(\omega_{eI}t) + 1\}\right] \tag{6}$$

Eq. (5) then provides

$$\tilde{H}^{S1,S2}_{int}(t) = 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{1}{2}\left(S_{1+}S_{2-}e^{-i\{\Delta\Omega t + \Xi(t)\}} + S_{1-}S_{2+}e^{i\{\Delta\Omega t + \Xi(t)\}}\right)\right] \tag{7}$$

The time-dependent exponents in this Equation can be expanded as

$$e^{i[\Delta\Omega t + \Xi(t)]} = e^{i\Delta\Omega t}\cdot e^{i2\pi J_{IS_1}\left(\frac{\Omega_I t}{\omega_{eI}} + \frac{\omega_{1I}}{\omega_{eI}^2}\right)}\cdot e^{i2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2}\right)\{\sin(\omega_{eI}t) - \cos(\omega_{eI}t)\}} \tag{8}$$

The recoupling effects in this experiment will arise when the oscillating terms in the exponential function, $\sin(\omega_{eI}t)$ and $\cos(\omega_{eI}t)$, originate time-dependencies that cancel out the oscillations imposed by $\Delta\Omega$. These terms can be found using the Bessel function expansion:

$$\begin{aligned} e^{i2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2}\right)\{\sin(\omega_{eI}t) - \cos(\omega_{eI}t)\}} &= \frac{e^{i2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2}\right)\sin(\omega_{eI}t)}}{e^{i2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2}\right)\cos(\omega_{eI}t)}} \\ &= \frac{\sum_{k=-\infty}^{\infty}J_k(z)\exp(ik\omega_{eI}t)}{\sum_{k=-\infty}^{\infty}(i)^kJ_k(z)\exp(ik\omega_{eI}t)} \end{aligned} \tag{9}$$

where z = $2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2}\right)$. Replacing this into Eq. (8), it follows that the time-dependencies averaging away the non-secular homonuclear terms will cancel out for numerous "decoupling sidebands" $k\omega_{eI}$. The most intense, k = 1, fulfills the condition

$$\Delta\Omega + 2\pi J_{IS_1}\left(\frac{\Omega_I}{\omega_{eI}}\right) + \omega_{eI} = 0 \tag{10}$$

leading to $e^{i[\Delta\Omega+\Xi(t)]t} \approx \frac{J_1(z)}{J_0(z)}$. Fulfilling this condition will in general require matching the S-spin chemical shift differences as

$$|\Omega_{2S} - \Omega_{1S}| = \frac{2\pi J_{IS_1}}{\sqrt{\omega_{1I}^2 + \Omega_I^2}}\Omega_I + \sqrt{\omega_{1I}^2 + \Omega_I^2} \approx \sqrt{\omega_{1I}^2 + \Omega_I^2} \tag{11}$$

Under such conditions, the average Hamiltonian for the recoupled S_1-S_2 interaction can be found by integrating Eq. (7) over the duration $t_p = 2\pi/\omega_{eI}$ of the I-spin nutation:

$$\begin{aligned}\overline{H_{int}^{S1,S2}} &\approx 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{J_1\left(\frac{2\pi J_{IS_1}\omega_{1I}}{\omega_{eI}^2}\right)}{J_0\left(\frac{2\pi J_{IS_1}\omega_{1I}}{\omega_{eI}^2}\right)}(S_{1+}S_{2-} + S_{1-}S_{2+})\right] \\ &\approx 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{\pi J_{IS_1}\omega_{1I}}{\omega_{eI}^2}(S_{1+}S_{2-} + S_{1-}S_{2+})\right]\end{aligned} \tag{12}$$

where we have used that when z is small, $J_0(z) \approx 1, J_1(z) \approx \frac{z}{2}$. Notice that this $\overline{H_{int}^{S1,S2}}$ has all the expected J,ω_{1I} and Ω_I dependencies adumbrated for a 2^{nd}-order effect.

With this analytical description as background, brute-force simulations were performed based on the numerical propagation of a system like the one just analyzed: a C_1-C_2 pair with distinct chemical shifts that are $\Delta\Omega$ = 1000 Hz apart, a J_{CC} = 50 Hz coupling among them, and a continuous irradiation of a proton that's only J-coupled to C_1. All the magnetization was assumed to start at C_1, and the $C_{1z} \rightarrow C_{2z}$ transfer was followed as a function of the ^1H RF field ω_{1H} and of the ^1H off-resonance frequency Ω_H. Fig. 1a depicts the dependence of the $C_{1z} \rightarrow C_{2z}$ transfer as a function of these variables, confirming a condition of maximum efficiency arising for $\Delta\Omega \approx \sqrt{\omega_{1H}^2 + \Omega_H^2}$, as predicted in Eq. (11). This homonuclear transfer is very efficient (Fig. 1b), and is achieved without pulsing on the ^{13}C; however, as can be noticed from Fig. 1a, its matching condition is also very narrow.

To increase the effective bandwidth of this HORRENDOUS transfer, we explored the strategy recently put forward in the AL FRESCO solid-state NMR method [25]: relying on a linearly chirped, undersampled ^1H pulse, in order to reintroduce the C_1-C_2 couplings. Under the effect of a chirp pulse irradiation applied to the I-spin, the relevant Hamiltonian is still as described in Eq. (1); the sole difference being the replacement of the last term, by:

$$\omega_{1I}I_x \rightarrow \omega_{1I}A(t)\left[I_x\cos\{\Psi(t)\} + I_y\sin\{\Psi(t)\}\right] \tag{13}$$

where $A(t)$ is the overall shape of the pulse and $\Psi(t)$ its phase profile. While in actuality a WURST-40 pulse was executed [33,34], for simplicity we assume here that $A(t) = 1$ and that the chirp's phase profile is given by:

$$\Psi(t) = \frac{BW}{2t_p}t^2 - \frac{BW}{2}t \tag{14}$$

where BW and t_p are the bandwidth and duration of the adiabatic chirped pulse, respectively, and for simplicity $\Psi(t)$ assumes that the pulse is centered at zero offset with zero initial phase. The chirp pulse's action on the I-spin can be accounted for using similar transformations as those leading from Eqs. (1) to (2), except that viewed in a frequency modulated (FM) frame specified by $UHU^\dagger + iU^\dagger dU/dt$, where $U = \exp[i\Psi(t)]$ [25,35,36]. This leads to a Hamiltonian that's analogue to that in Eq. (2)

$$\begin{aligned}H'_{FM} &= -\omega_{eI}(t)I'_z - \Omega_{1S}S_{1z} - \Omega_{2S}S_{2z} \\ &\quad + 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{1}{2}(S_{1+}S_{2-} + S_{1-}S_{2+})\right] \\ &\quad + 2\pi J_{IS_1}\left[\cos\theta_I(t)I'_z + \sin\theta_I(t)I'_x\right]S_{1z}\end{aligned} \tag{15}$$

except for the fact that the effective field and the tilt angles imposed by the *I*-spin RF, are now time-dependent:

$$\omega_{eI}(t) = \sqrt{\omega_{1I}^2 + \left[\Omega'_I(t)\right]^2} = \sqrt{\omega_{1I}^2 + \left(\Omega_I + \frac{BW}{2} - \frac{BW}{t_p}t\right)^2} \tag{16}$$

$$\cos\theta_I(t) = \frac{\Omega_I - \omega_p(t)}{\omega_{eI}(t)}; \sin\theta_I(t) = \frac{\omega_{1I}}{\omega_{eI}(t)}. \tag{17}$$

Here $\frac{d\psi(t)}{dt} = \omega_p(t)$ is the instantaneous frequency of the chirped pulse, and Ω_I is its center. Continuing with the same derivation as for the CW pulse above, one reaches an expression for $\overset{\sim S1,S2}{H_{int}}$ that

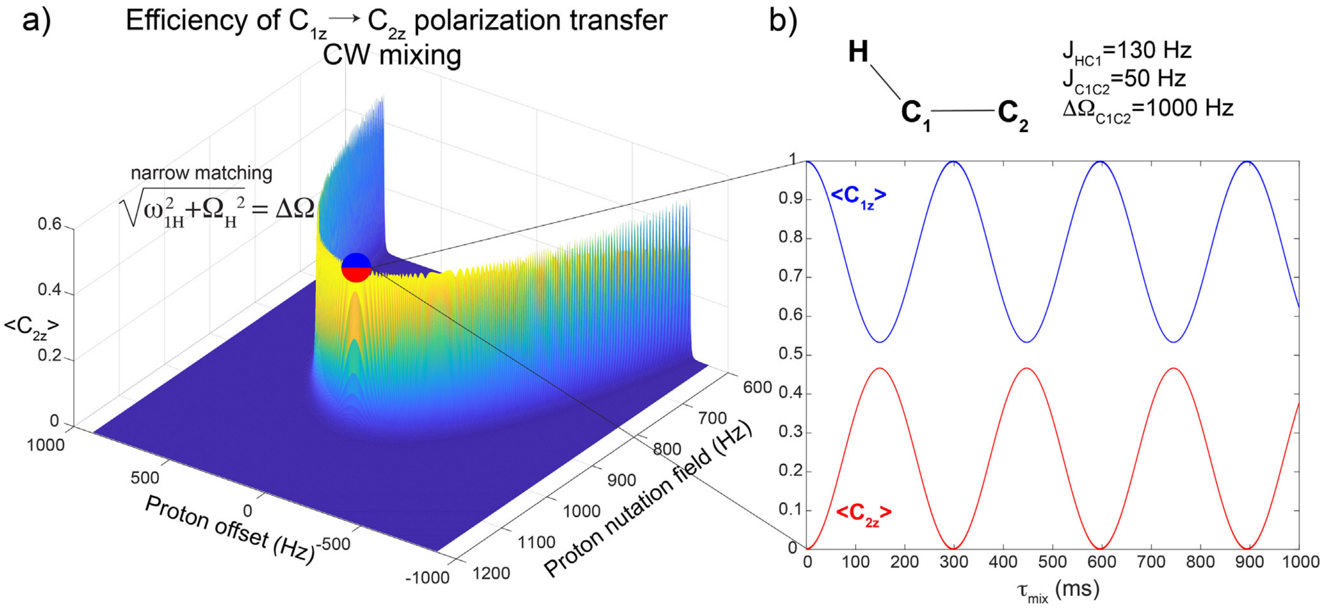

Fig. 1. (a) Maximum efficiency of a $C_{1z} \rightarrow C_{2z}$ transfer calculated by propagation of the Hamiltonian shown in Eq. (1), assuming CW ^1H decoupling and as function of the ^1H offset and ^1H nutation field. (b) Time evolution of the ^{13}C polarizations (and additional parameters as shown in the inset) for an on-resonance ^1H irradiation condition. As specified in the figure $J_{HC_1} = 130$ Hz; $J_{C_1C_1} = 50Hz$ and $\Delta\Omega_{C_1C_1} = 1000$ Hz.

is identical to that in Eq. (7), except that now the phase accrued as a result of the heteronuclear evolution will be given by

$$\Xi(t) = 2\pi J_{IS_1}\frac{1}{t} \times \int_0^t \left[\frac{\Omega_I - \omega_p(t_0)}{\omega_{eI}(t_0)} + \frac{\omega_{1I}}{\omega_{eI}(t_0)}\{\sin(\omega_{eI}(t_0)t) + \cos(\omega_{eI}(t_0)t)\}\right]dt, \tag{18}$$

where it is assumed that for a short period of time between t_0 and $t_0 + t$, quasi-static $\omega_{eI}(t_0)$ and $\omega_p(t_0)$ terms fulfilling the homonuclear S_1-S_2 recoupling have been met. Indeed, just as before, also in a frequency-modulated interaction representation this recoupling will arise if the time-dependencies imposed by chemical shift differences of the homonuclear flip-flop terms, $e^{\pm i\Delta\Omega t}$, interfere with the time-dependency of $\Xi(t)$–i.e., with the time dependency imposed by the RF on the heteronuclear coupling. This happens when the exponent in

$$e^{\pm i[\Delta\Omega t + \Xi(t)]} = e^{\pm i\Delta\Omega t} \cdot e^{\pm i2\pi J_{IS_1}\left(\frac{[\Omega_I - \omega_p(t_0)]t}{\omega_{eI}(t_0)} + \frac{\omega_{1I}}{\omega_{eI}^2(t_0)}\right)} \cdot e^{\pm i2\pi J_{IS_1}\left(\frac{\omega_{1I}}{\omega_{eI}^2(t_0)}\right)\{\sin(\omega_{eI}(t_0)t) - \cos(\omega_{eI}(t_0)t)\}} \tag{19}$$

becomes zero. Expanding the exponents in these trigonometric functions as Bessel functions (cf. Eq. (9)), the matching condition will happen when:

$$\Delta\Omega + 2\pi J_{IS_1}\left(\frac{[\Omega_I - \omega_p(t_0)]}{\omega_{eI}(t_0)}\right) + \omega_{eI}(t_0) = 0$$
$$\Longleftrightarrow |\Omega_{2S} - \Omega_{1S}| = \frac{2\pi J_{IS_1}\left[\Omega_I + \frac{BW}{2} - \frac{BW}{t_p}t_0\right]}{\sqrt{\omega_{1I}^2 + \left(\Omega_I + \frac{BW}{2} - \frac{BW}{t_p}t_0\right)^2}} + \sqrt{\omega_{1I}^2 + \left(\Omega_I + \frac{BW}{2} - \frac{BW}{t_p}t_0\right)^2} \tag{20}$$

This condition holds only for a specific instant in time during the adiabatic sweep, when $\omega_p(t) \approx \omega_p(t_0)$. Still, similar approximations as used above lead to an effective average Hamiltonian:

$$\overline{H_{int}^{S1,S2}} = 2\pi J_{S_1S_2}\left[S_{1z}S_{2z} + \frac{J_0[z(t_p)]J_1[z(t_p)]}{[J_0[z(t_p)]J_0[z(t_p)] + J_1[z(t_p)]J_1[z(t_p)]]}(S_{1+}S_{2-} + S_{1-}S_{2+})\right] \tag{21}$$

where $z(t_p) = 2\pi J_{IS_1}\omega_{1I}/\left[\omega_{1I}^2 + \left(\Omega_I + \frac{BW}{2} - \frac{BW}{t_p}t_0\right)^2\right]$.

The main benefit of using a chirped pulse is that the homonuclear recoupling will happen regardless of the *I*-spin offset Ω_I –as long as this offset falls within the bandwidth BW. Further, the RF fields ω_{1I} that will be required are still modest. The disadvantage of the chirped pulse is that the recoupling condition happens seldom, hence leading to a less efficient $S_1 \rightarrow S_2$ transfer. One way to make the chirp-driven transfer more efficient is to undersample its waveform, leading to periodic foldings of the swept RF. This enhances the transfer efficiency by repeatedly fulfilling Eq. (20), while preserving broadbandness [25]. Fig. 2 demonstrates this behavior for a three-spin system akin to that treated in Fig. 1, subject to a chirped mixing. As can be seen, choosing proper bandwidths and sampling dwells for this pulse can indeed lead to multiple contiguous recoupling modes, thereby making the $C_1 \rightarrow C_2$ transfers relatively immune to the ^{1}H offset. Notice also the incoherent-like nature that the multiply-folded chirp imparts on the ensuing transfer (Fig. 2b), while retaining an overall good efficiency.

With the aid of the undersampled chirped pulse this kind of recoupling is achievable not only for the simplest three-spin system, but also in four-spin systems of the kind commonly present in many larger molecules; here both carbons have directly bonded protons possessing different –and often *a priori* unknown– chemical shifts (Fig. 3). Good transfers can be achieved in both $C_1 \leftrightarrow C_2$ directions and, more importantly, for multiple proton chemical shifts; this is the kind of broadband transfer that would not be achievable using a CW irradiation. Notice as well that these transfers are effective even for relatively weak (e.g., 600 Hz) nutation fields.

3. Experimental results

Figs. 4 and 5 present applications of these principles to natural-abundance and to partially ^{13}C-enriched samples, respectively. Fig. 4 compares the results acquired on natural abundance thiophene upon inserting a HORRENDOUS chirp ^{1}H pulse during the mixing period of a 2D, NOESY-like ^{13}C–^{13}C correlation experiment –with and without any ^{1}H RF power (Fig. 4a and 4b, respectively). The ^{13}C–^{13}C correlation spectrum evidences strong cross-peaks between the ^{13}C satellites; no such cross-peaks are observed when the nutation field of the ^{1}H RF is set to zero. Fig. 5a compares a HORRENDOUS correlation recorded on a 25% ^{13}C-labeled glucose samples in water, against a TOCSY [7] counterpart recorded on the same sample with a DIPSI-2 sequence (Fig. 5b). Notice the low nutation fields that on the proton channel were needed for the mixing in both Figs. 4a and 5a; this compares to the nearly 1000-fold stronger power required for executing an efficient TOCSY transfer (Fig. 5b).

4. Discussion and conclusions

This study introduced a method capable of achieving ^{13}C–^{13}C transfers in liquids based on irradiating solely their J-coupled proton partners. The method is based on the application of a low nutation field ω_{1H} modulating the J_{CH} coupling, which under suitable conditions will partially reintroduce the C_1–C_2 J_{CC} flip-flop terms that were truncated away by a $\Delta\Omega >> J_{CC}$ offset. This can be rationalized by the decoupling sidebands introduced by proton irradiation, which when matching the separation between the ^{13}C spectral lines, will reactivate their non-secular J_{CC} contributions. As just described this HORRENDOUS scheme will be efficient but narrow-banded, something that was alleviated by the introduction of chirped ^{1}H pulses. To improve the efficiency of the ensuing scheme these swept pulses were undersampled, using clocking dwell times Δt that were longer than the inverse BW of the pulses; this causes multiple foldovers of the chirped pulse, equipping HORRENDOUS with broadbandness with respect to the protons' and carbons' offsets. This makes comparisons against TOCSY –which also delivers homonuclear correlations– natural; still, some clear differences between the two approaches are worth noting. The most evident one is that HORRENDOUS can transfer its correlations over timescales given by T_1, whereas TOCSY needs to operate on timescales given by $T_{1\rho}$. Moreover HORRENDOUS requires the presence of at least one heteronuclear J-coupling to perform a recoupling, whereas TOCSY does not. At a more subtle level HORRENDOUS's recoupling happens –even in instances involving the chirped ^{1}H irradiation– based on the sequential recoupling of many two-spin Hamiltonians; by contrast, TOCSY is driven by the sum of untruncated scalar J-coupling interactions acting simultaneously. This leads to TOCSY's complex multi-spin dynamics, often correlating with similar efficiency proximate and distant sites. The difference between this and the HORRENDOUS experiment, is reflected by the different patterns observed for the enriched glucose sample in Fig. 5. Still, it is conceivable that other, more systematic searches, can lead to better options for designing a ^{1}H RF irradiation scheme that reintroduces C–C non-secular terms, than the undersampled chirped pulses assayed in this study.

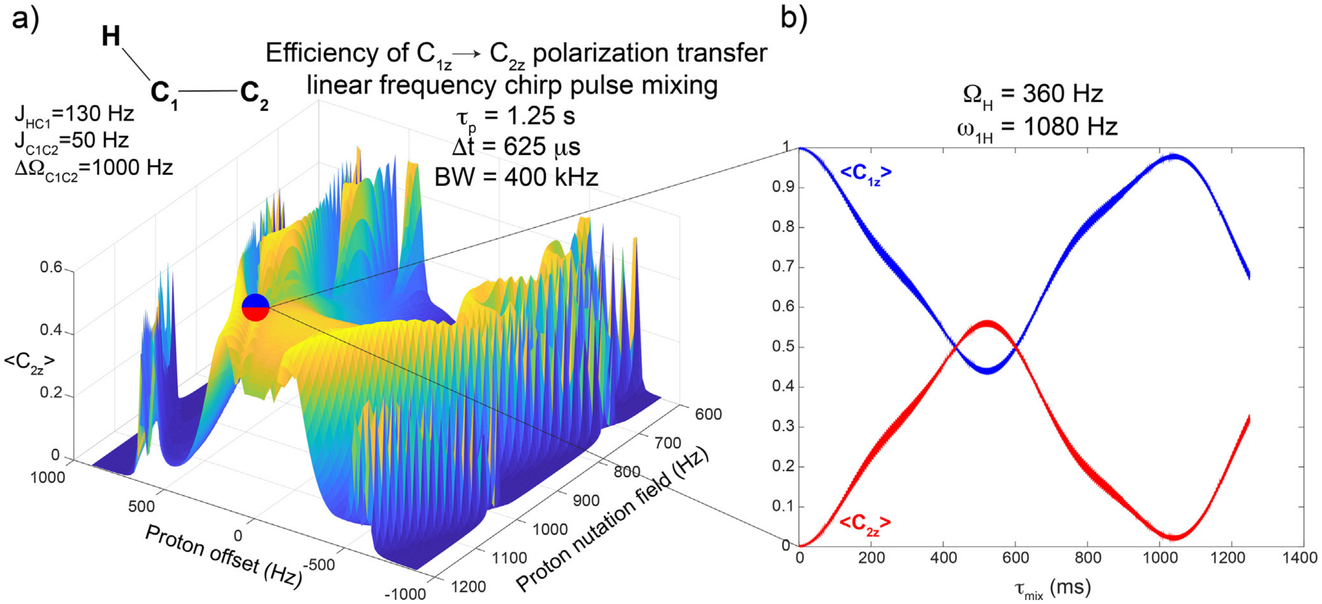

Fig. 2. Increasing the effective bandwidth of the HORRENDOUS transfer by adopting the AL FRESCO strategy [25], which relies on a linearly chirped, undersampled ^{1}H RF irradiation to reintroduce the C_1-C_2 couplings. Choosing proper bandwidths and sampling dwells for this pulse can lead to multiple contiguous recoupling modes, broadbanding the $C_1 \leftrightarrow C_2$ transfers introduced in Fig. 1. (a) Idem as Fig. 1a, for the same H-C_1-C_2 spin system but for ^{1}H pulses of the bandwidth, duration and clocking dwell as specified above the simulation. (b) Time evolution expected for the ^{13}C polarizations for the indicated ^{1}H offset and nutation field.

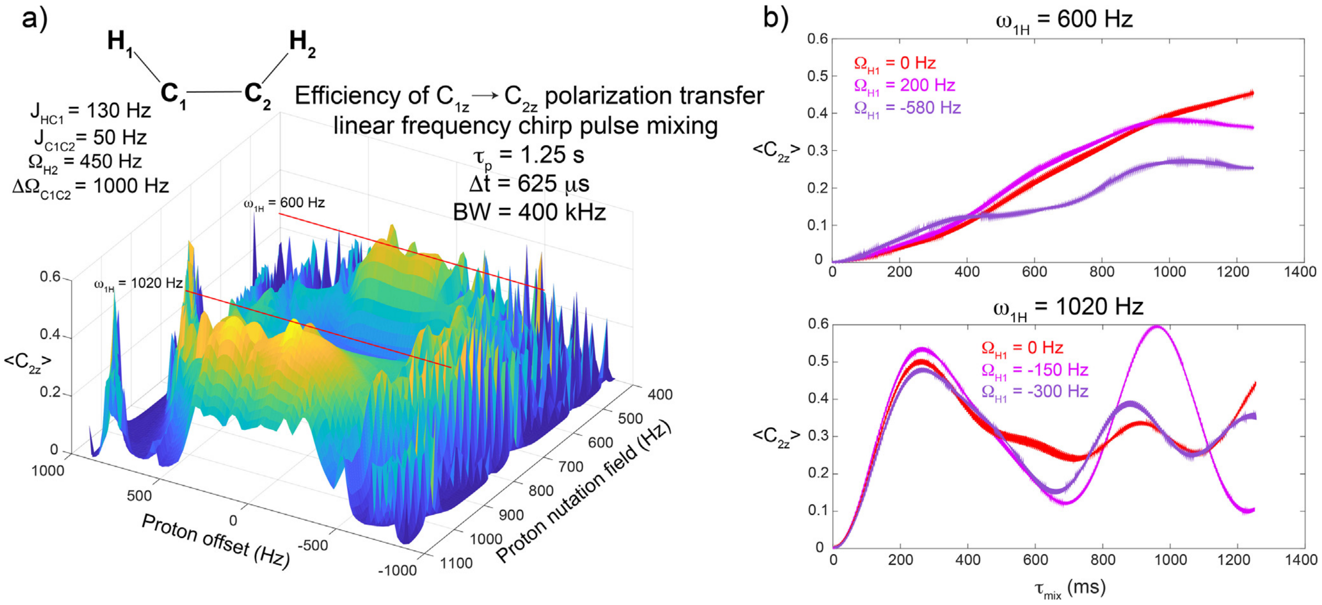

Fig. 3. (a) Maximum efficiency of a HORRENDOUS transfer relying on the same linearly chirped, underspamled ^{1}H RF irradiation as introduced in Fig. 2, for H_1-C_1-C_2-H_2 systems. In these calculations the offset of the first proton was varied, while for simplicity the offset of the second proton was kept fixed at $\Omega_H = 450$ Hz. (b) Examples of the C_2 magnetization buildups achieved for multiple ^{1}H offsets, for two different RF irradiation strengths (corresponding to the lines indicated in panel (a)).

When compared with solid-state NMR counterparts that rely on large homo- and hetero-nuclear dipolar couplings and enjoy multiple MAS-driven recoupling conditions, HORRENDOUS is faced by challenges and opportunities. On one hand all the J-couplings it involves are weaker, meaning lower transfer efficiencies. This will not be a limitation as long as the relaxation times T_1 of the ^{13}C are long, since no pulsing is needed on these species to implement their recoupling –their magnetization just “waits” along the z-axis while the latter happens. Slow transfers, however, may be a limitation for larger molecules with shorter T_1s –which will still probably be longer than their $T_{1\rho}$. On the other hand, the method has the advantage of not requiring large RF fields, as it does not have to overcome large dipolar couplings nor the large offsets characteristic of ^{13}C or other heavy nuclides: in fact, its power deposition requirements seem to be lower than those of spin-lock-based analogues. Potential further applications of this HORRENDOUS principle –including better recoupling schemes, combinations of longitudinal and transverse transfers, and correlations among nuclei other than ^{13}C– are being explored.

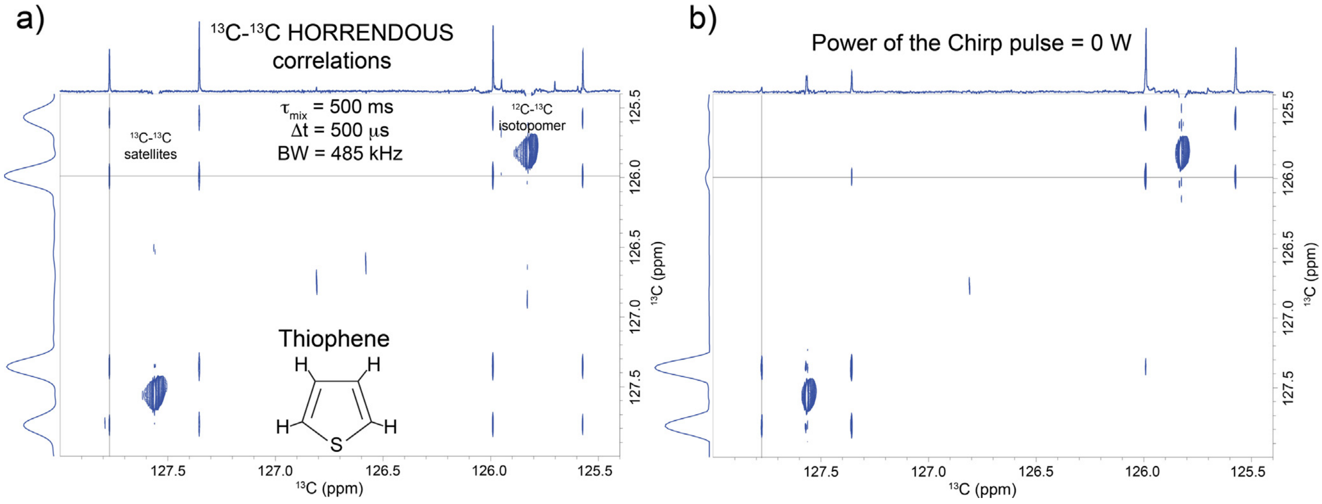

Fig. 4. (a) Experimental ^{13}C–^{13}C correlation spectra achieved using a ^{1}H chirp pulse on natural abundance thiophene. Cross-sections extracted at the carbon satellites (top, left) illustrate the efficient polarization transfers between the ^{13}C isotopomers, upon using a 500 ms mixing and a 530 Hz ^{1}H nutation field. (b) Same experiment but acquired upon turning off the power of the ^{1}H chirp pulse; notice the absence of ^{13}C–^{13}C correlations (left projections). Spectra were recorded on a 600 MHz Bruker Avance III spectrometer equipped with a Prodigy probe.

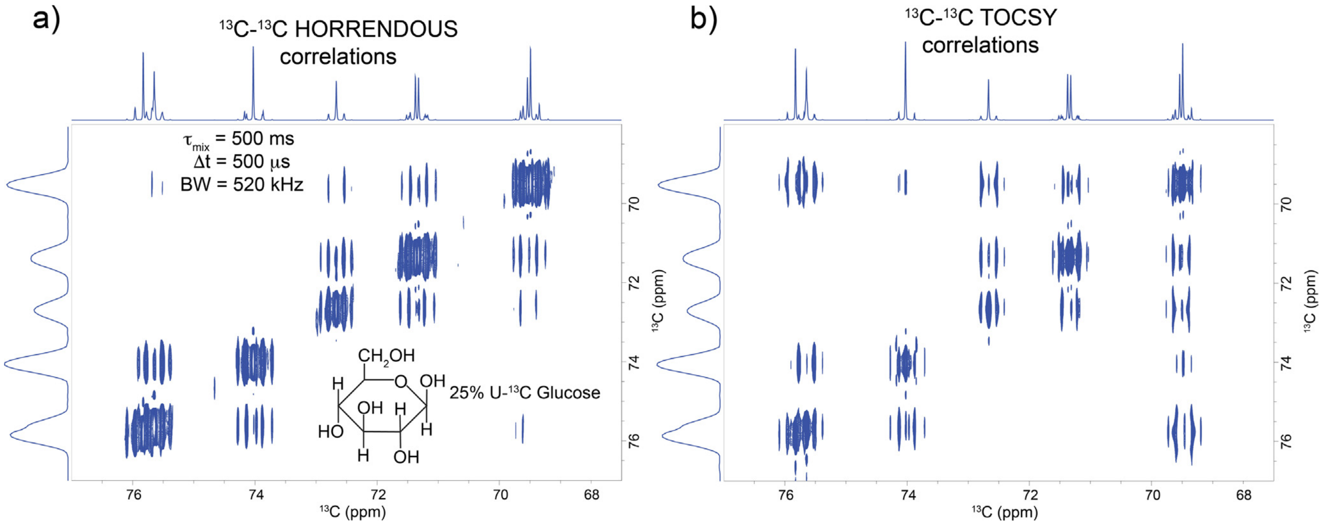

Fig. 5. Experimental ^{13}C–^{13}C correlation spectra achieved using: (a) a HORRENDOUS ^{1}H chirped mixing pulse with the mentioned mixing, clocking-out and bandwidth parameters, and (b) a conventional ^{13}C DIPSI2 spin-lock irradiation, on 25% ^{13}C-labeled glucose. In (a) a 900 Hz nutation field involving 6.5 mW deposited on the probe was used, compared to an almost 8 W ^{13}C DIPSI2 irradiation for the TOCSY experiment in (b). Both experiments used the same mixing period (0.5 sec). Spectra were recorded on a 600 MHz Bruker Avance III spectrometer equipped with a Prodigy probe.

5. Materials and methods

5.1. Sample preparation

Thiophene was purchased from Acros Organics and prepared as a 90% solution with CDCl$_3$ solvent that is added for locking. D-glucose (U–^{13}C$_6$, 24–25%) was purchased from Cambridge Isotope Laboratories and a 100 mM sample was prepared using D$_2$O as a solvent.

5.2. NMR spectroscopy

NMR experiments were conducted on a 14.1 T (600 MHz) Avance III Bruker spectrometer equipped with a liquid-nitrogen-cooled "Prodigy" probe. The HORRENDOUS pulse program was written as a variant of Bruker standard dipsi2ph sequence, where the mixing period was changed to include a proton chirped pulse instead of the carbon–carbon DIPSI2 sequence. HORRENDOUS mixing chirped pulses were generated using Bruker's ShapeTool® according to simulation predictions. As an example, for thiophene, a 4-spin HCCH spin system was used with couplings and chemical shifts values extracted from ^{1}H and ^{13}C 1D spectra; the chirped pulse duration, bandwidth and nutation field, were then optimized in the simulation to yield a 50% transfer efficiency. Examples of these sequences are provided in the Supporting Information. All spectra were processed in TopSpin® 3.6.1 using QSINE window function, zero-filling (four times) and Fourier transform and T$_1$ noise suppression. All pulse sequences are available from the authors upon request. In order to verify that ensuing ^{13}C–^{13}C correlations are generated as a result of proton irradiation –even though the tested samples were in the fast-tumbling regime, where NOE cross-peaks are easily discernible from J-driven correlations by their sign– reference experiments were acquired with power of chirp pulse set to 0 W which yielded no correlations at all (Fig. 4). The TOCSY spectrum in Fig. 5b was acquired using DIPSI2 [7] isotropic mixing within standard Bruker dipsi2ph experiment. Thiophene experiments were acquired with 128 F_1 points (53 ms t_1 evolution times); experiments on glucose were acquired using 256 points and a 14 ms total t_1 evolution time. These relatively short evolution times lead to the elongated F_1 line shapes appearing in these spectra.

5.3. Simulations

All numerical simulations are carried out using home-written Matlab® (The MathWorks Inc.) codes based on product operator formalism that involved numerical propagation of density-matrix evolution under the effect of proton irradiation. The simulations did not include relaxation effects; these codes are available in the Supporting Information.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmr.2022.107176.

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