Supplementary Information for

Revealing signs and hidden ¹H-NMR coupling constants in threespin systems using Long-Lived Coherences

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PS 1. Long-Lived Coherences (LLC)

Sarkar et al.¹ showed that, in a two-spin system, the lifetime of the coherence between the singlet state, $|S_0\rangle = |\alpha\beta - \beta\alpha\rangle/\sqrt{2}$, and the central triplet state, $|T_0\rangle = |\alpha\beta + \beta\alpha\rangle/\sqrt{2}$,

$$Q_{LLC} = |S_0\rangle\langle T_0| + |T_0\rangle\langle S_0|, \tag{S1}$$

is long-lived under favorable circumstances. The LLC is a zero quantum coherence in the rotating frame and is written in the product-operator basis as

$$\rho_{LLC} = I_z - S_z \tag{S2}$$

The lifetime of this coherence, called a Long-lived Coherence (LLC), is $T_{LLC} = (R_{LLC})^{-1}$, where $R_{LLC} = \left(\frac{b_{LS}^2}{20}\right)[j(0) + 3j(\omega) + 6j(2\omega)] + R_{LLC}^{ext}$. Contributions from external random fields, anisotropic chemical shifts, and dipole-dipole interactions to remote spins form R_{LLC}^{ext} . The transverse relaxation time, T_2 , is three (small molecules) to nine times (macromolecules) smaller than T_{LLC} , when these other factors are comparable.¹

PS 2. Pulse Sequence

The coherence in equation S2 can be created by one of the four pulse sequences shown in Figure S1.² In all these sequences, the filled rectangles represent a high power $\pi/2$ -pulse and the unfilled rectangles, smaller in amplitude, represents a relatively low power continuous locking pulse. The power of this pulse maybe 5-10 times of the chemical shift difference between two peaks in a two-spin system.

Pulse sequence in Figure S2 (a) is used in most cases. A $\pi/2$ pulse converts the Boltzmann population into a single quantum coherence, $-I_y - S_y$, which after evolving for $\tau = 1/2\Delta v$ is converted to $I_x - S_x$.³ Locking with a continuous wave (CW) pulse in the *x*-direction provides $I_z - S_z$. Chemical shift evolution is also suppressed during locking resulting in the magnetization evolving only under the coupling Hamiltonian, i.e., $2\pi J I_1$. I_2 . As a result, the LLC in a two-spin system oscillates at the coupling constant, *J*. It decays with a relaxation time of the order of three to nine times that of the transverse relaxation time T_2 , i.e., $\frac{T_{LLC}}{T_2} = 3 \text{ to } 9.^4$ This oscillating decay resembles the Free Induction Decay (FID), but because of it being observed during a sustaining pulse, it is known as Sustained Induction Decay (SID).²



Figure S1. All four pulse sequences create $I_x - S_x$, which corresponds to coherence between singlet and central triplet state before spin-locking with a continuous pulse. The τ_1 is chosen to be $1/(2\Delta\nu)$, where $\Delta\nu$ = chemical shift difference between two chemically non-equivalent protons.²

PS 3. Materials and methods

All NMR experiments were performed on Bruker Avance-III HD spectrometers equipped with broadband probes and operating at ¹H NMR frequencies of 400 MHz or 500 MHz.

Solutions of 2-furoyl chloride, ethyl vinyl ether, and 2, 3-dibromopropionic acid in deuterated chloroform (CDCl₃), and acrylic acid, phenylalanine, and bispidinone in deuterated water (D₂O) were used for the experiments.

Reactions to synthesize bispidinone were carried out in oven-dried glassware. Reagents were purchased from Sigma-Aldrich, CDH (India). Commercial chemicals were used without further purification. All solvents were dried by standard methods. Progress of reactions was monitored by silica gel thin-layer chromatography (TLC). Compounds were purified by silica gel (100-200 mesh) column chromatography. **Scheme S1:** Synthesis of Compound **A4**.⁵



Reagents (a) Benzyl amine, HCHO, AcOH, MeOH, 65^{0} C, 10h, 60% (b) TFA, DCM, rt, 4h (c) H₂/10% of Pd/C, MeOH, AcOH, rt, 8h

Further details of the synthetic procedure are given in Haridas et al.⁵

The ¹H-NMR spectrum of bispidinone

¹H-NMR (D₂O, 500 MHz); δ 2.37(br s, 2H), 3.44 (br d, 4H), 3.62 (dd, *J*=13.85, 4.45 Hz, 4H)



Compounds	Chemical Structures				
2-furoyl chloride (FC)					
Acrylic acid	Н Н Н				
2,3-dibromopropionic acid (DBPA)					
Ethyl vinyl ether (EVE)	H H H OCH ₂ CH ₃				
Phenylalanine (PA)	H H COOH H ₂ N				

Table S1: Chemical Structures of the compounds used for the study

The three protons forming *J*-coupling network for all three-spin compounds are highlighted in the chemical structures.

PS 4. LLC spectrum of a two-spin system

The LLC spectrum shown in Figure S2 (b) is obtained by Fourier Transformation of the Sustained Induction Decay (SID), shown in Figure S2 (a), which is mapped point-by-point as the spin-lock period is incremented. From Figure S2 (b) it is clear that in the case of two spin systems, the LLC spectrum shows a peak at the coupling constant $(\pm J)$, however, for convenience of representation only the peak at +J is displayed in the LLC spectrum, Figure S2(c). Only the positive part of the LLC spectrum is displayed throughout the paper.



Figure S2. (a) Sustained Induction Decay (SID) of 2, 3-dibromothiophene (DBT). (b) LLC Spectrum of DBT obtained from Fourier Transformation of SID. (c) Expansion of LLC spectrum to present the coupling information conveniently.

PS 5. LLC frequencies in a three-spin system

In contrast to a two-spin system, the peaks in the LLC spectrum of a three-spin system do not occur at the coupling constant frequencies. Instead, the coherences oscillate at differences of the eigenvalues of the *J*-coupling Hamiltonian during spin locking.

The Hamiltonian under consideration for an A, K, and X system of three strongly coupled spins is⁶

$$\hat{H} = J_{AK}\hat{I}_{A}.\,\hat{I}_{K} + J_{KX}\hat{I}_{K}.\,\hat{I}_{X} + J_{AX}\hat{I}_{A}.\,\hat{I}_{X},$$
(S3)

This is rewritten as

$$\widehat{H} = J\left(\widehat{I}_A.\,\widehat{I}_K + a(\widehat{I}_K.\,\widehat{I}_X) + b(\widehat{I}_A.\,\widehat{I}_X)\right),\tag{S4}$$

by defining $J_{AK} = J$, $a = \frac{J_{KX}}{J_{AK}}$, $b = \frac{J_{AX}}{J_{AK}}$. In the product basis this has the form

$$\begin{pmatrix} \frac{J}{4}(1-a-b) & \frac{aJ}{2} & \frac{bJ}{2} \\ \frac{aJ}{2} & -\frac{J}{4}(1+a-b) & \frac{J}{2} \\ \frac{bJ}{2} & \frac{J}{2} & -\frac{J}{4}(1-a+b) \end{pmatrix} = 0.$$
(S5)

What we have shown above is the Hamiltonian in the m = +1/2 manifold. A similar matrix results for the m = -1/2 manifold. The eigenvalues of the Hamiltonian are

$$E_1 = \frac{J}{4}(1+a+b)$$
(S6)

$$E_2 = -\frac{J}{4}(1+a+b) + \frac{J}{2}(1+a^2+b^2-a-b-ab)^{\frac{1}{2}}$$
(S7)

$$E_3 = -\frac{J}{4}(1+a+b) - \frac{J}{2}(1+a^2+b^2-a-b-ab)^{\frac{1}{2}}$$
(S8)

The coherences in a three-spin system oscillate at the frequencies given by pair-wise differences of the eigenvalues in the m = +1/2 or m = -1/2 manifolds.⁷

$$\nu_1 = J(1 + a^2 + b^2 - a - b - ab)^{\frac{1}{2}}$$
(S9)

$$\nu_2 = \frac{J}{2} \left[(1+a+b) + (1+a^2+b^2-a-b-ab)^{\frac{1}{2}} \right]$$
(S10)

S7

$$\nu_3 = \frac{J}{2} \Big[(1+a+b) - (1+a^2+b^2-a-b-ab)^{\frac{1}{2}} \Big]$$
(S11)

PS 6. Comparison between experimental and simulated LLC spectra

LLC spectra of 2-furoyl chloride, acrylic acid, 2, 3-dibromopropionic acid, ethyl vinyl ether, and phenylalanine were recorded with the procedure described in section 4. The observed LLC peak positions were matched with simulated values using the coupling constants determined from the ¹H-NMR spectrum of the respective compounds. The simulations were performed on MATLAB R2015a. The initial density matrix obtained after a $\pi/2$ -pulse and a delay of $1/2\Delta \nu$, where $\Delta \nu$ is the chemical shift difference between two peaks whose average was chosen as the carrier frequency, was propagated with the isotropic mixing Hamiltonian, $2\pi J(I_A.I_K + I_A.I_X + I_K.I_X)$. The expectation value of the operator $I_{AX} + I_{KX} + I_{XX}$ provides the SID for three-spin systems. The SIDs for various compounds are Fourier transformed to obtain the simulated LLC spectrum for the three-spin systems. The experimental and simulated LLC spectra are shown in Figures S(3) to Figure S(8). The sharp edges in the LLC spectra are due to small number of points. Acquiring more points will result in extended experimental time. Despite the poor resolution, which results in sharp edges, the peak positions are easily identified. The experimental spectrum of bispidinone shows two peaks. The position of the third peak is determined by the relation defined by the equation $v_1 = v_2 - v_3$. The simulated spectrum was obtained after finding the unknown coupling constant using the procedure detailed in the manuscript and therefore shows all peaks.



Figure S3. (a) Experimental and (b) Simulated LLC spectrum of 2-furoyl chloride.



Figure S4. (a) Experimental and (b) Simulated LLC spectrum of acrylic acid.







Figure S6. (a) Experimental and (b) Simulated LLC spectrum of ethyl vinyl ether.



Figure S7. (a) Experimental and (b) Simulated LLC spectrum of phenyl alanine.



Figure S8. (a) Experimental and (b) Simulated LLC spectrum of bispidinone.

Table S2 given below compares the experimental and simulated LLC spectra of all compounds except bispidinone.

Three spin	Three spinExperimentalCalculated peaks for various signsystemL values (incombinations (Hz)					Observed LLC peaks (in Hz)	Simulated
Hz)							Hz)*
2-furoyl chloride	3.69(±0.07)	1.89	0.32	-0.96	-1.98	1.85	1.85(±0.09)
	1.66(±0.07)	2.51	3.95	3.75	3.07	2.50	2.55(±0.08)
	0.85(±0.07)	4.40	4.27	4.71	5.05	4.35	4.45(±0.07)
Acrylic acid	17.31(±0.04)	7.43	5.23	-8.16	-9.43	7.43	7.45(±0.043)
	10.53(±0.04)	14.09	16.14	16.05	15.09	14.15	14.15(±0.04)
	1.11(±0.04)	21.52	21.43	24.22	24.53	21.64	21.55(±0.04)
2,3-dibromo propionic acid	11.23(±0.05)	6.40	0.97	-6.72	7.94	6.50	6.75(±0.054)
	10.13(±0.05)	9.65	15.06	12.17	-11.19	12.22	12.25(±0.062)
	4.35(±0.05)	16.05	16.03	18.88	19.13	18.73	18.95(±0.049)
Ethyl vinyl ether	14.36(±0.07)	6.11	2.60	-4.52	-6.79	2.27	2.65(±0.068)
	6.84(±0.07)	10.87	14.09	13.94	12.42	13.83	14.15(±0.07)
	-1.90(±0.07)	16.98	16.69	18.45	19.21	16.11	16.75(±0.07)
Phenylalanine	14.7(±0.06)	8.36	-0.076	-3.97	-10.12	9.36	10.15(±0.07)
	8.1(±0.06)	9.87	17.57	15.87	11.42	11.54	11.45(±0.07)
	5.3(±0.06)	18.23	17.65	19.85	21.54	20.9	21.55(±0.06)
			1	1	1	1	1

 Table S2: Comparison of the experimental and the simulated LLC spectrum to find out a relative sign of the coupling constants

PS 7. References

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