Electronic Supplementary Information

SABRE-SHEATH hyperpolarized $^{15}N_2$-imidazole for Zn^{2+} sensing

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1. Materials

Standard SABRE pre-catalyst [IrCl(COD)(IMes)] (where, IMes = 1,3-bis(2,4,6-trimethyl-phenyl)imidazole-2-ylidene) was synthesized by following a previously established procedure.[1] HPLC-grade methanol was obtained from Fisher Chemical, biotech-grade sodium hydroxide pellets from Spectrum Chemical, ACS-grade hydrochloric acid from EMD Millipore Corporation, and extra-pure zinc(II) chloride from Thermo Fisher. Additionally, $^{15}N_2$-imidazole was synthesized by following a previously established procedure[2] and high purity *p*-H_2 (> 93%) was produced using a previously reported system.[3] A SevenDirect SD23 pH / conductivity meter with InLab Micro ProISM instrument was used to perform pH measurements and a 1.4 T Magritek benchtop NMR spectrometer (model: Spinsolve 60 Multi X) with ^{15}N nuclei capability was used to acquire the ^{15}N NMR spectra.

2. Detailed experimental procedure, data acquisition and processing

2.1 Experimental procedure

A sample containing 100 mM $^{15}N_2$-imidazole, 6 mM Ir-IMes pre-catalyst and 1 mM NaOH in methanol was prepared, and a 0.8 mL aliquot of the solution was carefully transferred into an NMR tube (See main paper, Figure 2). The hyperpolarization pre-catalyst in the sample was then activated by flowing *p*-H_2 at a rate of 100 sccm and a pressure of 100 psi at 52 °C for 10 minutes. Once fully activated, the sample changed color from pale yellow to colorless. The activated sample was transferred to a mu-metal shield, where *p*-H_2 was bubbled for 40 seconds at an optimized polarization transfer field of 0.4 µT and a temperature of 37 °C to hyperpolarize the ^{15}N nuclei of $^{15}N_2$-imidazole (See SI, section 8 for the optimization studies of polarization build-up, polarization-transfer field and temperature). After hyperpolarization, the sample was transferred to a 1.4 T Magritek benchtop NMR spectrometer and depressurized by disconnecting the NMR sample tube from the bubbling setup. Immediately a 30-degree pulse was applied to acquire a proton-decoupled ^{15}N NMR signal of neutral $^{15}N_2$-imidazole. Finally, the analyte of interest (Zn^{2+} solution, or HCl solution, or methanol for the control experiment) was injected into the NMR tube using a syringe. The solutions were mixed by shaking the PTFE catheter, and a 30-degree pulse was applied to acquire the proton-decoupled ^{15}N NMR signal to detect the chemical interactions.

For HCl titration (See main paper, Figure 3), a 100 mM stock solution of HCl was prepared in methanol. Varying volumes of this stock were injected into separate, depressurized samples to achieve the final concentrations of 2.4, 9.1, 13, 20, 33.3 and 42.9 mM of the mixtures as shown in the main paper.

For $ZnCl_2$ titration (See main paper, Figure 4), a 100 mM primary stock solution of $ZnCl_2$ was prepared and then serially diluted to create stock solutions of 100, 50, 25, 12.5, 6.3, 3.1, and 0.8 mM. For each experiment, a 150 µL aliquot of the corresponding stock solution was injected into the depressurized sample to reach the final concentration of 15.8, 7.9, 3.9, 2, 1, 0.5, 0.1 mM of the mixture as shown in the main paper.

As a control, an 80 µL aliquot of pure methanol was injected into the depressurized sample to account for any effects from solvent addition and dilution. This control measurement represents the starting point of the HCl and $ZnCl_2$ titrations (0 mM analyte), as shown by the black traces in Figures 3 and 4 of the main paper.

2.2 Data acquisition

All ^{15}N NMR spectra were acquired on a 1.4 T Magritek Spinsolve 60 Multi X benchtop spectrometer using Spinsolve software (v. 2.1.3.5116). The protocol details are described below:
Protocol name: 1D NITROGEN+ WALTZ
Protocol details:

Pulse Angle = 30
Number of points = 8192
Bandwidth = 822 ppm
Center Frequency = 150 ppm
Noe Enhancement = no
Decouple = yes (namely, WALTZ)
Receiver Gain = 31

2.3 Data processing

All the acquired ^{15}N NMR spectra were processed using MestReNova software (v. 15.0.1-35756).
Processing details:

Apodization = basic exponential, 1Hz
Zero filling = from original FID size 8192 to 16384
Baseline Correction = no
Phase correction = automatic

3. ^{15}N NMR spectrum of SABRE-SHEATH hyperpolarized $^{15}N_2$-imidazole over a larger bandwidth

The SABRE-SHEATH hyperpolarized ^{15}N NMR spectrum of $^{15}N_2$-imidazole in methanol is shown in Figure S1. Hyperpolarized free peak displayed identical chemical shifts of 203.4 ppm for both ^{15}N nuclei, attributed to rapid proton hopping between the two nitrogen sites (See main paper, Figure 1 for the structure). The additional small, hyperpolarized peaks correspond to Ir-bound $^{15}N_2$-imidazole species at various positions within the $^{15}N_2$-imidazole hexacoordinate Ir complex. Although a definitive assignment of these bound peaks was not pursued, it's noteworthy that the number and positions of these hyperpolarized bound peaks are identical to those previously reported by Roman et al. for SABRE-SHEATH hyperpolarized $^{15}N_2$-imidazole.[4]

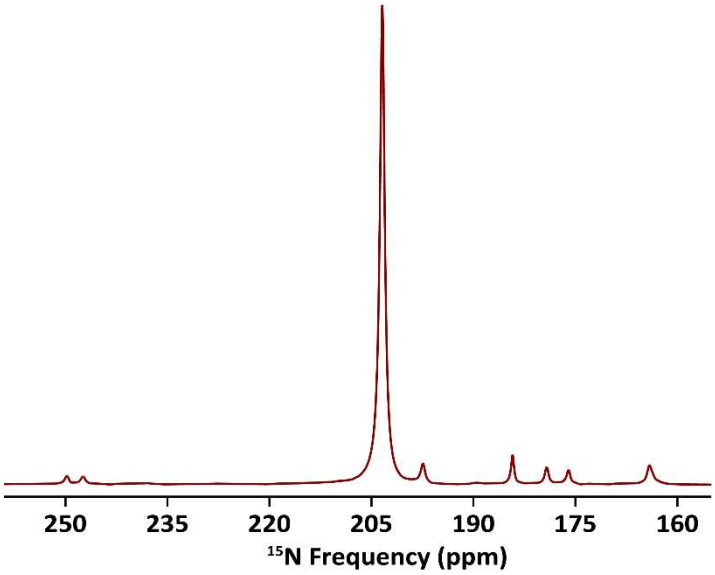

Figure S1. SABRE-SHEATH hyperpolarized proton-decoupled ^{15}N NMR spectrum of $^{15}N_2$-imidazole acquired using a 1.4 T Magritek benchtop NMR spectrometer.

4. Calculation of enhancement and polarization

The percentage of polarization for the hyperpolarized $^{15}N_2$-imidazole was calculated using the thermal spectrum of a reference sample, specifically, neat ^{15}N-pyridine thermalized at 1.4 T. The calculation is explained below:

Enhancement = $\left(\frac{SD_{Ref}}{SD_{HP}} \times \frac{CA_{Ref}}{CA_{HP}} \times \frac{SI_{HP}}{SI_{Ref}}\right)$

Percentage of polarization = $\% P_{thermal}$ × Enhancement

Here,

$\% P_{thermal} = \tanh\left(\frac{\hbar \times \gamma_{15N} \times B_0}{2 \times k_B \times T}\right) \times 100\%$

where $\hbar$ = reduced Planck's constant, γ_{15N} = gyromagnetic ratio of ^{15}N nucleus, B_0 = magnetic field strength, k_B = Boltzmann constant, T = temperature in Kelvin.

SD_{HP} = spin density of hyperpolarized substrate
SD_{Ref} = spin density of reference sample
where, spin density = (concentration × the number of ^{15}N nuclei contributed to the signal)

CA_{HP} = cross – sectional area of hyperpolarized substrate
CA_{Ref} = cross – sectional area of reference sample

SI_{HP} = integrals of the signal intensity of hyperpolarized substrate
SI_{Ref} = integrals of the signal intensity of reference sample

4.1 ^{15}N NMR spectrum of the reference sample (neat ^{15}N-pyridine thermalized at 1.4 T)

Thermal spectrum of neat ^{15}N-pyridine thermalized at 1.4 T was used as a reference to calculate the percentage of polarization for the hyperpolarized $^{15}N_2$-imidazole.

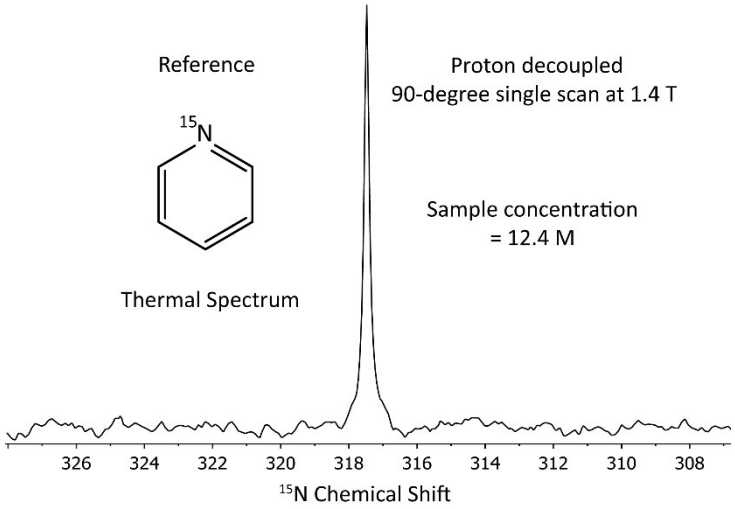

Figure S2. Proton-decoupled ^{15}N NMR spectrum of a thermally polarized neat ^{15}N-pyridine reference sample at 1.4 T.

4.2 The ratio of CA_{Ref} to CA_{HP} determination

The reference ^{15}N-pyridine scan was conducted in a 5 mm NMR tube without a capillary, while the hyperpolarized sample utilized a similar 5 mm NMR tube fitted with a capillary for introducing *p*-H_2.

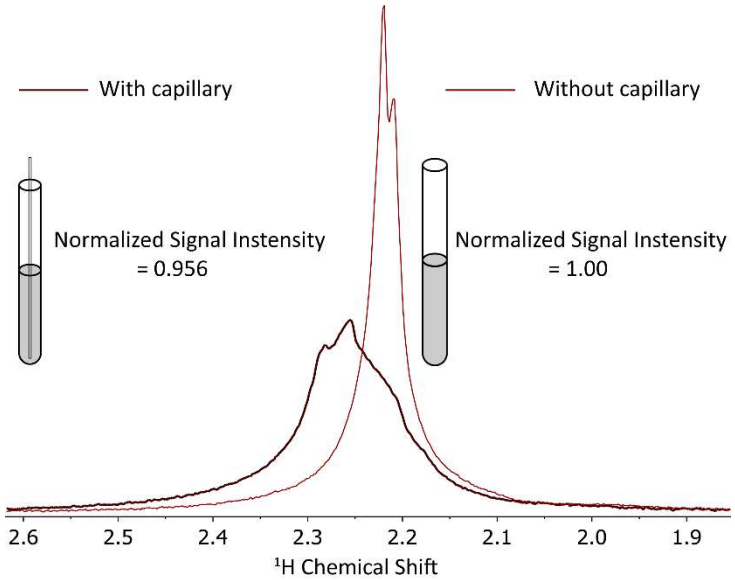

Figure S3. A 90-degree single ^{1}H NMR scan of 0.91M acetonitrile (CH_3CN) in chloroform ($CDCl_3$), performed in a 5 mm NMR tube with and without a capillary tube.

A value of 1.046 for the ratio of CA_{Ref} to CA_{HP} was obtained from Figure S3.

4.3 A representative calculation for this work

The spin density for this work was calculated as follows:

$SD_{Ref} = (12.4\ M\ \times 1) = 12.4\ M$

$SD_{HP} = \left(0.1 - (3\ \times 0.006)\right)\ M\ \times 2 = 0.164\ M$

where an initial concentration of 100 mM $^{15}N_2$-imidazole and 6 mM catalyst was used throughout the experiment to prepare the sample. We assumed 3 substrates are linked to the catalyst resulting in a free substrate concentration of 82 mM and a bound substrate concentration of 18 mM. Free hyperpolarized $^{15}N_2$-imidazole spectrum contains 2 equivalents of ^{15}N nuclei signal due to the rapid proton hopping of neutral $^{15}N_2$-imidazole structure.

Table S1. Enhancement and polarization calculation.

γ_{15N}	2.71E7 rad·s^{-1}·T^{-1}
$\hbar$	1.06E-34 J·s
B_0	1.4 T
k_B	1.38E-23 J·K^{-1}
T	310 K
SI_{Ref}	15.35
SI_{HP}	8872.61
$\frac{CA_{Ref}}{CA_{HP}}$	1.046
SD_{Ref}	12.4 M
SD_{HP}	0.164 M

% $P_{thermal}$	4.70E-5
Enhancement	45714
% of polarization	2.15

5. pKa measurement for $^{15}N_2$-imidazole in methanol

The pKa of $^{15}N_2$-imidazole in methanol was determined by volumetric titration. This involved titrating 5 mL of 0.1 M $^{15}N_2$-imidazole (weak base) with 0.1 M HCl (strong acid), as depicted in Figure S4. The midpoint of this titration curve occurred at a pH of ≈ 5.8, representing the pKa value.

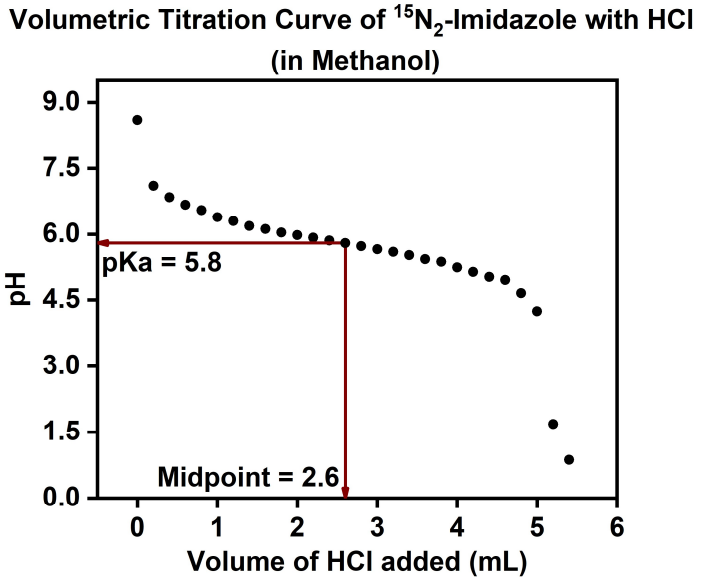

Figure S4. Volumetric titration curve of $^{15}N_2$-imidazole in methanol with HCl. A 5 mL sample of 0.1 M $^{15}N_2$-imidazole was titrated with 0.1 M HCl. A sharp decrease in pH from 4.2 to 1.7 was found at 5.2 mL of HCl addition indicated the equivalence point. Accordingly, the midpoint of the titration was identified at 2.6 mL of HCl addition, corresponding to a pH of ≈ 5.8, which represents the pKa of $^{15}N_2$-imidazole in methanol.

6. pH calculation using Henderson-Hasselbalch equation and comparison with experimental measurements

A SevenDirect SD23 pH / conductivity meter with InLab Micro ProISM instrument was used to perform pH measurements. The pH for the titration of $^{15}N_2$-imidazole with HCl (See main paper, Figure 3) was also calculated following Henderson-Hasselbalch equation and compared with the measured pH values.

Henderson-Hasselbalch equation can be described as follows:

$$pH = pKa + \log \frac{[\mu mol\ of\ base]}{[\mu mol\ of\ acid]}$$

Here,
pKa of $^{15}N_2$-imidazole ≈ 5.8 (From Figure S4)
μmol of base = (final concentration of $^{15}N_2$-imidazole in the mixture in mM) × (volume of the total mixture in mL)
μmol of acid = (final concentration of HCl in the mixture in mM) × (volume of the total mixture in mL)

Table S2. Comparison of measured and calculated pH for the titration of $^{15}N_2$-imidazole with HCl (See main paper, Figure 3).

$^{15}N_2$-imidazole in the mixture (μmol)	HCl in the mixture (μmol)[#]	Calculated pH	Measured pH
80	59.2	5.9	5.8
80	39.2	6.1	6.0
80	19.2	6.4	6.2
80	11.2	6.7	6.5
80	7.2	6.8	6.7
80	1.2	7.6	7.7

[#]The amount of HCl (in μmol) present in the mixture was calculated by subtracting the μmol of NaOH added during sample preparation from the μmol of HCl introduced into the hyperpolarized sample, assuming a 1:1 molar reaction between NaOH and HCl.

7. Limit of detection (LOD) calculation

Fitting table for the calibration curve of $^{15}N_2$-imidazole with Zn^{2+} (See main paper, Figure 5) is given below:

Table S3. Fitting table for the calibration curve of $^{15}N_2$-imidazole with Zn^{2+} (See main paper, Figure 5).

Y = mX +c (linear equation)	
Slope (m)	-0.49 ± 0.02
Intercept (c)	203.3 ± 0.1
Adj. R^2	0.984
Pearson's r	-0.994

Peak center of the $^{15}N_2$-imidazole, y_b = 203.4 ppm
Intercept of the calibration curve, c = 203.3
Slope, m = -0.49

$\delta = \frac{\mathrm{FWHM}}{2}$ = 0.25 ppm

LOD = $\frac{(y_b - c) - (3\times\delta)}{m} = \frac{(203.4-203.3)-(3\times0.25)}{-0.49}$ = 1.3 mM

8. SABRE-SHEATH optimization studies

8.1 Sample preparation for optimization studies

Given that $^{15}N_2$-imidazole hyperpolarizes more effectively under basic conditions,[4] sample preparation for the SABRE-SHEATH optimization studies were conducted under basic conditions. This was achieved by adding 10 mM NaOH during sample preparation. Accordingly, the sample composition for the SABRE-SHEATH optimization studies included 100 mM $^{15}N_2$-imidazole, 6 mM catalyst, and 10 mM NaOH in a 1 mL methanol solution. A 0.8 mL aliquot of this sample was transferred to the NMR tube and experiments were conducted for the optimization studies. However, for the zinc sensing (See main paper, Figure 4) and HCl titration (See main paper, Figure 3), the NaOH concentration was reduced to 1 mM in the sample to prevent the formation of $Zn(OH)_2$ and to carry out a direct comparison between them, as reported in the main paper.

8.2 Polarization build-up study

The experimental procedure for the SABRE-SHEATH hyperpolarization of $^{15}N_2$-imidazole sample is described in the main paper (See main paper, Figure 2b). A study on polarization build-up was conducted to determine the optimal *p*-H_2 bubbling time for achieving polarization saturation. This involved bubbling ≈ 93% *p*-H_2 for varying durations before signal acquisition at 1.4 T.

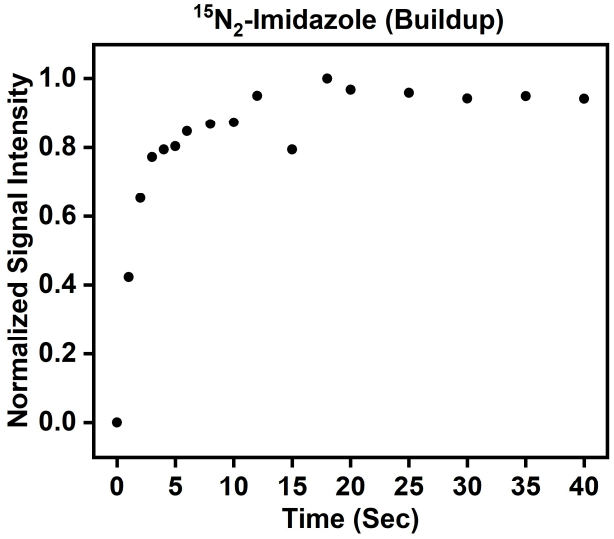

Figure S5. SABRE-SHEATH hyperpolarization build-up study of $^{15}N_2$-imidazole revealed polarization saturation after 25 seconds of bubbling with ≈ 93% *p*-H_2.

8.3 Polarization transfer field optimization study

The optimal magnetic field for polarization transfer from *p*-H_2 to the ^{15}N nuclei of $^{15}N_2$-imidazole was then investigated. This involved subjecting the sample to various low fields (μT) inside a mu-metal shield via a solenoid (See main paper, Figure 2b for the setup), followed by bubbling with ≈ 93% *p*-H_2 for 40 seconds prior to signal acquisition at 1.4 T.

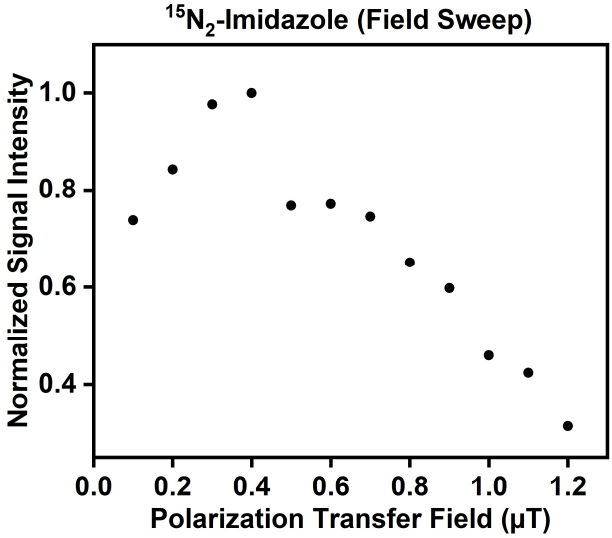

Figure S6. Polarization transfer field optimization study demonstrates that 0.4 μT is the optimal field for transferring polarization from *p*-H_2 to the ^{15}N nuclei of $^{15}N_2$-imidazole.

8.4 Temperature optimization study

A temperature optimization study was performed by placing the sample tube in a water bath at various temperatures inside the mu-metal shield. For these experiments, a 0.4 μT polarization transfer field was applied and ≈ 93% *p*-H_2 was bubbled through the sample for 40 seconds prior to signal acquisition at 1.4 T (See main paper, Figure 2b for the setup).

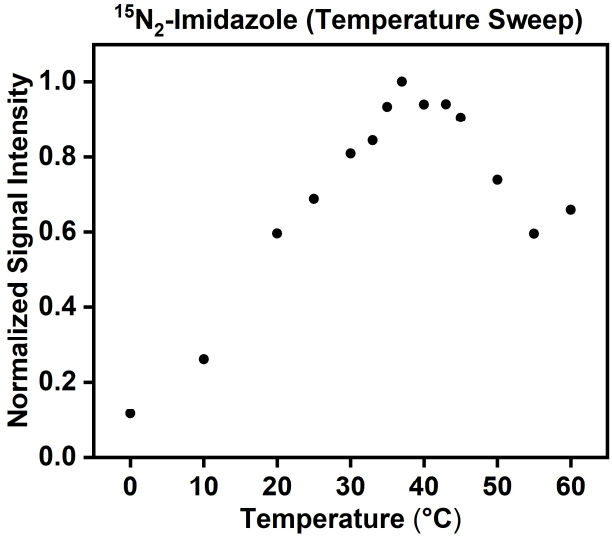

Figure S7. Temperature optimization study indicates that 37°C is the optimal temperature for hyperpolarizing $^{15}N_2$-imidazole.

8.5 Longitudinal (T_1) relaxation study

To measure the T_1 relaxation time, the sample was first hyperpolarized under optimized conditions, i.e., at a polarization transfer field of 0.4 μT and temperature of 37°C by bubbling with ≈ 93% *p*-H_2 for 40 seconds. The hyperpolarized sample was then transferred to 1.4 T Magritek benchtop NMR spectrometer and stored for varying time before signal acquisition. The proton-decoupled ^{15}N NMR signal was acquired by applying a 90-degree pulse.

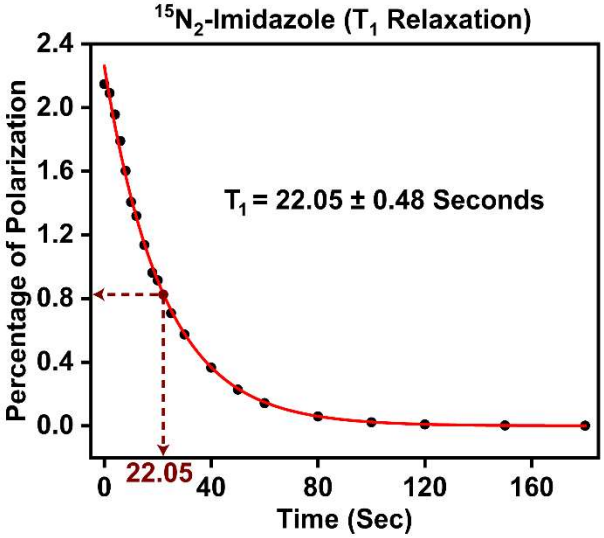

Figure S8. Relaxation study of the ^{15}N nuclei of $^{15}N_2$-imidazole at 1.4 T magnetic field shows a T_1 relaxation time of 22.05 ± 0.48 seconds.

9. References for SI

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