# The Unified Scale for Referencing in NMR: New IUPAC Recommendations

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In 2001, IUPAC set new definitions and standards for NMR referencing,[1] and updated these in 2008.[2] A significant change from past conventions is the introduction of a "unified scale," with a single primary reference of the $^{1}H$ resonance of TMS for ***all*** nuclei. The unified scale relies on $\Xi$ values,[3] stated as percentages:[4]

$$\Xi_X \equiv 100 \times \left( \nu_X \,/\, \nu_{TMS}^{obs} \right) \qquad (1)$$

where $\nu_X$ = the absolute frequency for the 0 ppm position in the *X* spectrum, and $\nu_{TMS}^{obs}$ = the absolute frequency for $^{1}H$ of TMS.

Eq. (1) has been used extensively for referencing spectra of proteins and nucleic acids.[5] In particular, eq. 1 is used to reference the indirectly observed $^{13}C$ and/or $^{15}N$ dimensions of 2D and nD spectra.

Eq. (1) has also found widespread use for referencing *X* nuclei, where direct detection of that nucleus's secondary reference[6] is difficult. One example would be $^{57}Fe$: the low natural abundance and gyromagnetic ratio makes detection of the standard difficult. Another example would be $^{199}Hg$, where the former primary reference, dimethyl mercury, is too toxic to recommend its use.

It is important to note that the IUPAC has recommended that the unified scale used for referencing ***all nuclei***, including such common nuclei as $^{31}P$, $^{13}C$, and $^{29}Si$.

## Recommendations:

1. Although some spectra should continue to be referenced as in the past, a significant number should change to follow the new recommendations. It is recommended that faculty review the referencing procedures used in their groups.
2. State clearly in publications whether the unified scale is being used for referencing or not:
   - If the unified scale is used, include complete reference information and the $\Xi_X$ value used.
   - If the unified scale was not used, ***always provide*** $\nu_{TMS}^{obs}$***, or as good a substitute as available*** (e.g., $\nu_{^{31}P}^{obs}$ with clear statements about how 0 ppm was determined for the $^{31}P$ spectrum).
3. The fact that δ is a ***measured*** quantity is stressed, with its intrinsic dependence on the experimental conditions. It is therefore important to describe the environment of the sample and reference compound by listing:
   - use of the internal, external, or substitution method;
   - the solvent, concentration of solute and internal reference, and temperature.

## Practical Considerations

### $^1H$ Spectra:

Referencing $^1H$ spectra should follow past guidelines and considerations. IUPAC makes a point of not encroaching on many traditional aspects for NMR referencing, in particular those that have real practical value.[7]

*Internal Referencing:* Use an internal standard that is suitable with the solvent/solute system. Although 1% TMS in $CDCl_3$ is the recommended primary standard, TMS in other solvents or other concentrations is acceptable, as well as other secondary standards, or where necessary external or substitution referencing.

*External Referencing:* This method is discouraged when possible with respect to the previous and following methods, as a susceptibility correction must be applied; otherwise reference as usual. Consideration should be made whether substitution referencing would be preferable to the external method.

*Substitution Referencing:* Adjust properly for any changes in solvent, then reference as described for internal referencing. It is likely that the best substitution reference is a $^1H$ spectrum of TMS at ~1% in the same solvent as your compound.

### *X* Spectra:

Using a suitable secondary reference compound is no longer recommended. The unified scale is the preferred method of referencing all spectra.

***Example:*** In the past, the middle peak of the $CDCl_3$ 1:1:1 triplet at 77.0ppm was often used to reference $^{13}C$ spectra. *[NOTE: If you reference using a secondary standard such as $CDCl_3$, you should still provide $\nu_{TMS}^{obs}$ in publications.]*

<u>The recommended method for $^{13}C$ spectral referencing is the following:</u>

1. Obtain a $^1H$ spectrum of the same compound, and set 0 ppm using the TMS resonance.
2. Determine $\nu_{TMS}^{obs}$ from the $^1H$ spectrum (see below for details).
3. Use $\nu_C = \nu_{TMS}^{obs} \frac{\Xi_C}{100}$ , where $\Xi_C = 25.145020$

   to determine the absolute frequency of the 0 ppm position in the $^{13}C$ spectrum.
4. Use $\nu_C$ as shown below to reference the $^{13}C$ spectrum.

## NUTS 1D Spectra

When using our Varian spectrometers, the simplest method for implementing the unified scale is to properly reference the spectrum using the **xref** macro in VNMR as described below, prior to saving the spectrum and moving it into NUTS.

For Bruker data, NUTS can straightforwardly perform absolute referencing, as follows:

1. Acquire $^{1}H$ and *X*-nucleus data from the compound during the same session (same lock, same shims). Save as the raw data on the spectrometer for importing into NUTS.
2. Work the data up as normal in NUTS, and properly reference the $^{1}H$ spectrum. Save both as NUTS spectra.
3. With the $^{1}H$ spectrum properly referenced and open in NUTS, run the appropriate macro using **RU**. The facility provides the following macro:

   absref_13C.mac
   absref_19F.mac
   absref_31P.mac

   All are simple modifications of the example described in AcornNMR's on-line documentation. Macros for other nuclei can easily be made.
4. Open the X-nucleus (NUTS) spectrum as requested by the macro. This spectrum will be properly referenced by the macro.

## Bruker AC Spectra

[The simplest procedure is to use NUTS as described above.]

$\nu_{TMS}^{obs} = SF_H$ from the properly referenced $^{1}H$ spectrum.
Set $SR_X = \nu_X - SF0_X$ in the *X* spectrum.

For example:

An organic compound run at 0.1M in $CDCl_3$ with 0.1% TMS was run on the Bruker AC-300+ (Athena). The $^{1}H$ spectrum properly referenced gave:

$\nu_{TMS}^{obs} = SF_H = 299.8727928$ MHz [ = SF0 + SR = 299.870 MHz + 2792.8 Hz]

A $^{13}C$ spectrum then is referenced using eq. 1:

$$\nu_C = \nu_{TMS}^{obs} \times \Xi_C / 100 = 299.8727928 \times 25.145020 / 100 = 75.4030737 \text{ MHz}$$

$SR_C = \nu_C - SF0_C = 75.4030737 - 75.410$ MHz $= -6926.3$ Hz in the unified scale.

Setting the center peak in $CDCl_3$ equal to 77.0 ppm as a alternative secondary reference,
$SR_C = -6919.3$ Hz

## Bruker Avance Spectra

[The simplest procedure for 1D data is to use NUTS as described above. ]

$\nu_{TMS}^{obs} = SF_H = BF1_H + SR_H$ from the properly referenced $^{1}H$ spectrum.
Set $SR_X = \nu_X - BF1_X$ in the *X* spectrum.

For example:

An organic compound run at 0.1M in $CDCl_3$ with 0.1% TMS was run on the Bruker AVANCE-360. The $^1H$ spectrum properly referenced gave:

$\nu_{TMS}^{obs} = SF_H = 360.1300157$ MHz [ = BF1 + SR = 360.130 MHz + 15.7 Hz]

A $^{13}C$ spectrum then is referenced using eq. 1:

$$\nu_C = \nu_{TMS}^{obs} \times \Xi_C / 100 = 360.1300157 \times 25.145020 / 100 = 90.5547645 \text{ MHz}$$

$SR_C = \nu_C - BF1_C = 90.5547645 - 90.555$ MHz = –235.5 Hz in the unified scale.

Setting the center peak in $CDCl_3$ equal to 77.0 ppm as a alternative secondary reference,

$SR_C$ = –235.5 Hz

## Varian VNMR Spectra

A figure written to assist in understanding such calculations in VNMR is provided at:
http://www.chem.wisc.edu/~cic/nmr/Guides/VUG/absolute_referencing_in_VNMR.pdf

For 1D *X* spectra:
a) Acquire a $^1H$ spectrum and reference it properly.
b) In another experiment, acquire the *X* spectrum, and use the macro **xref**.

For 2D *X* spectra:
a) Properly reference the $^1H$ axis (assumed F2/channel 1); copy **rfl** and **rfp** if necessary from a referenced 1D $^1H$ spectrum.
b) If the indirect dimension is from channel 2, use the macro **decref**.
If the indirect dimension is from channel 3, use the macro **decref2**.

For 3D spectra:
a) Properly reference the $^1H$ axis (assumed F3/channel 1); move **rfl** and **rfp** if necessary from a referenced 1D $^1H$ spectrum.
b) Use the macro **decref** to reference the indirect dimension F1 (channel 2 is assumed).
c) Use the macro **decref22** to reference the indirect dimension F2 (channel 3 is assumed).

[Varian has greatly updated their software for such calculations; see their documentation of the macro **mref** (described under **setref**). Note that these new definitions are not compatible with older UNITY system software, such as used with our 500; thus we have stayed with the older, facility-customized macros as defined above.]

1. R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, R. Goodfellow, and P. Granger, "NMR Nomenclature. Nuclear Spin Properties and Conventions for Chemical Shifts (IUPAC Recommendations 2001)", Pure and Applied Chemistry 73, 1795-1818 (2001). The paper is available on-line at: http://www.iupac.org/publications/pac/2001/7311/7311x1795.html
2. R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, P. Granger, R.E Hoffman, K.W. Zilm, "Further conventions for NMR shielding and chemical shifts," *Pure Appl. Chem.* **80**, 59-84 (2008). Available at: http://www.iupac.org/publications/pac/80/1/0059. Primary updates include:
   (a) $\Xi$ values from Ref 1 are re-listed in Appendix 1, with one correction and updates including: $\Xi_{Helium}$ is corrected; $\Xi_{DSS}$ is provided for various nuclei where the solvent might be $D_2O/H_2O$ (especially for studies of proteins and nucleic acids in aqueous solutions). This listing of $\Xi$ values is recommended to never be changed in the future (i.e., the values are to be considered accurate enough to now be immutable).
   (b) The temperature dependence of TMS is discussed. No correction need be made for temperatures close to 25°C. A value of $-5\times10^{-4}$ ppm/K should be used to correct the chemical shift of TMS for large temperature variations between –20 to +80°C; for more details, see Ref. 2 and citations 9,10 therein.
   (c) Bulk magnetic susceptibility effects are described in detail. For homogeneous samples (i.e., in most cases), this effect is identical, and no susceptibility measurement or correction is needed. See Ref 2 when performing external referencing, or other cases where susceptibility variations are present.
   (d) The utility of TMS is substantiated, due to its reasonable independence from changes in concentration, pressure, and temperature when conditions are: concentration ≤ 1%, pressure ~ 1 atm, and temperature ~ 25°C. IUPAC stresses the need to specify whenever these conditions are significantly different, as well as specifying the solvent, the solute concentration, and clearly stating when the sample has been deoxygenated. The effects of external referencing are significant, and this should always be described in detail.
   (e) Definitions are extended to solid-state NMR, including the recommended symbols σ for shielding, and δ for chemical shift.
3. Ref 2, Appendix 1.
4. Ref 1, eq. 9.
5. J. L. Markley, A. Bax, Y. Arata, C. W. Hilbers, R. Kaptein, B. D. Sykes, P. E. Wright, K. Wüthrich. "Recommendations for the Presentation of NMR Structures of Proteins and Nucleic Acids," *Pure Appl. Chem.* **70**, 117 (1998).
6. $^1H$ of TMS is the ***only*** primary reference compound in the unified scale. What formerly would have been denoted as a primary reference compound for a nucleus *X* is now simply called the *X* secondary reference. An *X* nucleus can have alternative secondary references: see section 3.7 of Ref 1.
7. Ref. 1: see section 3.4, the 3rd and 4th paragraphs of pg. 1810, and the Appendix.