

## Automatic heteronuclear referencing with `setref`

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A series of macros has been installed on the Varian instruments to accurately reference heteronuclear 1D, 2D or 3D spectra. These macros follow the recommendations given by IUPAC (1) regarding the reference of NMR spectra.

IUPAC recommends the use of a universal internal standard (“the primary reference”) to reference the spectra of *all* nuclei. This is the  $^1\text{H}$  signal of TMS in dilute solution. From its absorption frequency, the chemical shift of any other nucleus in the sample can be calculated and the corresponding scale can be set based on secondary references currently in use in the literature. The use of inconvenient or dangerous internal or external standards is thus eliminated. In aqueous solution the primary internal standard is DSS [sodium 3-(trimethylsilyl)propane-1-sulfonate] for solubility reasons. If no internal standard is present, the spectrum can be referenced using the frequency of the deuterated solvent (the lock frequency).

The macros are used as follows.

Measure and reference a proton spectrum as is normally done with VNMR. If you did this in experiment 1 and need a  $^{31}\text{P}$  spectrum, go to another experiment, for example experiment 2 (`jexp2`) and take your spectrum. The command `mref(1,2)` transfers (moves) the reference from experiment 1 to experiment 2. Your  $^{31}\text{P}$  is now referenced to internal TMS and is equivalent to using the conventional 85%  $\text{H}_3\text{PO}_4$  external standard but without its inconveniences.

For a 2D spectrum, first reference the observe (or F2) dimension, *i.e.* proton for all indirect detection experiments like HMQC, HMBC, etc. The command `reff1` then calculates the correct reference for the second (F1) dimension based on the proton reference.

Alternatively, if there is no TMS or DSS in the sample, the solvent's  $^2\text{H}$  absorption frequency can be used. Once the sample is locked, the macro `setref` calculates the chemical shift scale for the nucleus of interest based on the deuterium chemical shift of the solvent. Notice that you have not referenced your sample to TMS after calling `setref`, but you have simply *estimated* the reference frequency based on an estimated chemical shift value for the lock signal. Remember also that the chemical shift for many NMR solvents (such as  $\text{D}_2\text{O}$ ,  $\text{CDCl}_3$ ) is subject to changes in temperature, pH / pD, solute interaction, etc. The macros `setref1` and `setref2` do the same thing as `setref` but operate on the f1 and f2 dimensions of 2D and 3D spectra.

### Notes:

For some nuclei, notably  $^{15}\text{N}$  and  $^{31}\text{P}$ , there may be more than one standard (secondary reference) in use in the literature. These macros calculate the chemical shift based on the secondary references recommended by IUPAC for use in organic chemistry, for example:

<sup>13</sup> C	TMS
<sup>15</sup> N	CH <sub>3</sub> NO <sub>2</sub>
<sup>19</sup> F	CCl <sub>3</sub> F
<sup>31</sup> P	H <sub>3</sub> PO <sub>4</sub>
<sup>11</sup> B	BF <sub>3</sub> ·Et <sub>2</sub> O / CDCl <sub>3</sub>
<sup>113</sup> Cd	Me <sub>2</sub> Cd (neat)
<sup>195</sup> Pt	Na <sub>2</sub> PtCl <sub>6</sub> / D <sub>2</sub> O, 1.2 M
<sup>199</sup> Hg	Me <sub>2</sub> Hg (neat)

The complete list of standards used can be found in the lab computers in /vnmr/nuctables/nuctabref (open a terminal in Linux and type the command “less /vnmr/nuctables/nuctabref”).

### For more information,

- (1) R.K. Harris, E.D. Becker, S.M. Cabral de Menezes, R. Goodfellow, & P. Granger, "NMR Nomenclature. Nuclear Spin Properties and Conventions for Chemical Shifts (IUPAC Recommendations 2001)", *Pure and Applied Chemistry* 73, 1795-1818 (2001). <http://www.iupac.org/publications/pac/2001/7311/7311x1795.html>
- (2) In any spectrometer: /vnmr/nuctables/nuctabref
- (3) In any spectrometer: /vnmr/solvents
- (4) The macros' manual pages in vnmrj [man('setref'), man('mref'), etc].
- (5) “The unified scale for referencing in nmr. New IUPAC recommendations”. [http://www.chem.wisc.edu/~cic/nmr/Guides/Other/Xi\\_chem\\_shift\\_scale.pdf](http://www.chem.wisc.edu/~cic/nmr/Guides/Other/Xi_chem_shift_scale.pdf)
- (6) “Sigma vs. delta chemical shift scales in nmr”. [http://www.chem.wisc.edu/~cic/nmr/Guides/Other/sigma-vs-delta\\_chem\\_shift\\_scales.pdf](http://www.chem.wisc.edu/~cic/nmr/Guides/Other/sigma-vs-delta_chem_shift_scales.pdf)