

## Standard Operating Procedure

**Task:** Absolute Referencing

**Date:** 08/29/18

### Background:

- Absolute referencing is an internally consistent and accurate method to reference the chemical shifts of spectra of NMR active heteronuclei using TMS is used as a universal standard. Absolute referencing is the method recommended by IUPAC, replacing previous methods that relied on external standards for chemical shift referencing. This methodology improves reproducibility of chemical shift referencing without requiring the use of any external standards.

### Training Requirements:

- Lab safety training and any specific training for sample preparation.
- NMR spectrometer training

### Potential Hazards:


- Many NMR solvents are hazardous themselves and can carry dissolved materials through the gloves into the skin. Be aware of hazards associated both solvents and dissolved materials.
- Magnetic Field Warning! Older magnets have large stray fields; avoid bringing magnetic materials into the fields. Avoid strong fields if you have metal implants or a pacemaker.

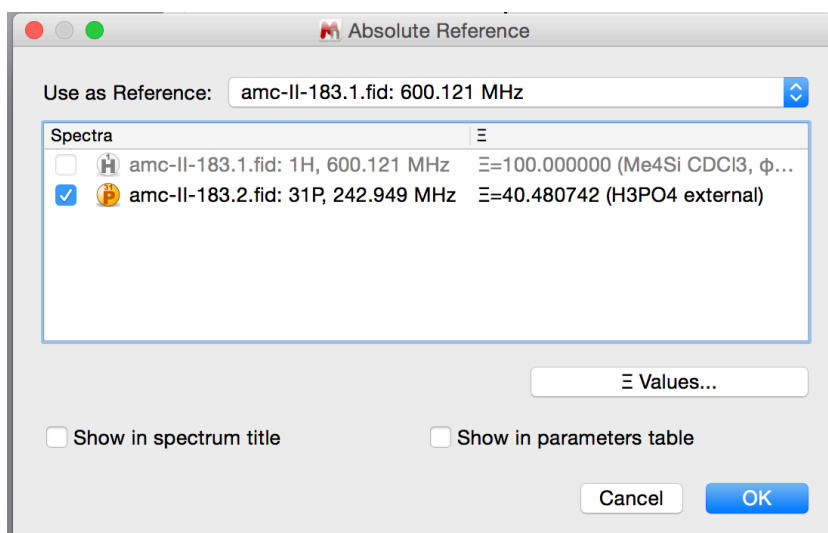
### Materials Needed:

- NMR tube
- Deuterated solvent (or a secondary reference if protio solvent will be used)
- NMR spectrometer

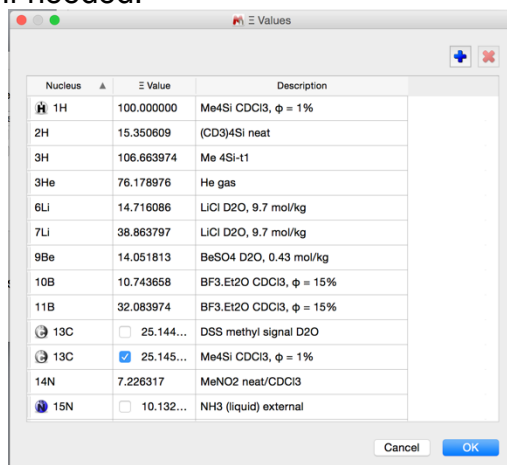
### Procedure:

#### *Method Setup*

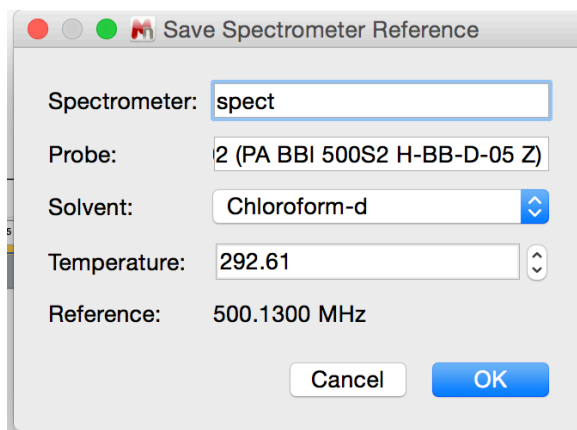
1. Prepare an NMR sample in deuterated solvent. Or, if the solvent does not have a well-defined solvent residual resonance, include a secondary reference such as hexamethyldisiloxane, ferrocene, mesitylene, etc.
2. Lock, shim, and tune on your sample as normal.
3. Acquire a  $^1\text{H}$  NMR spectrum.
4. Tune the spectrometer to any heteronuclei of interest and acquire a spectrum. This can be repeated for any heteronuclear experiments, including 2D spectra. NMR spectra of each type of nucleus acquired i.e.  $^{31}\text{P}$  vs  $^{13}\text{C}$ ) will require the spectrometer to be tuned.  
**Note: An external reference is not required to accurately reference heteronuclei in absolute referencing!**
5. Import data to Mestrenova.
6. Reference the 1D proton spectrum relative to TMS using the known chemical shift relative to TMS of the deuterated solvent residual (or secondary standard).
7. Click the absolute referencing button on Mestrenova .
8. This will pull up the Absolute Referencing windows:




9. At the top of the window, a pull-down menu contains a list of experiments loaded into the MNOVA file. Select the referenced proton spectrum from step 6.
10. Check any experiments to be referenced; multiple experiments can be referenced at the same time from the referenced proton.
11. Xi values ( $\Xi_x$ , see below) are automatically included in Mestrenova; additional  $\Xi$  values can be added manually if needed.



12. Acquiring a  $^1\text{H}$  NMR spectrum with an internal standard **every** time spectra of heteronuclei are acquired is **highly** recommended. However, if this is impractical, references can be saved from a prior proton acquisition and be applied to spectra where a proton was not acquired directly before X-nuclei acquisition. **This is spectrometer, solvent, and temperature dependent and may drift over time!**
  - Click Analysis>Reference>Save Reference



- Click the arrow on the Absolute Reference Button .
- Select “Apply Saved Reference”
- Your spectrum is now referenced!

### 13. To view saved references:

- Analysis>Reference>Edit Saved References

### Related SOPs:

- Non-uniform Sampling

### Further information and Resources:

- Harris, R. K.; Becker, E. D.; Cabral De Menezes, S. M.; Goodfellow, R.; Granger, P. NMR Nomenclature: Nuclear Spin Properties and Conventions for Chemical Shifts (IUPAC Recommendations 2001). *Concepts Magn. Reson. Part A Bridg. Educ. Res.* 2002, 14, 326–346.
- Harris, R. K.; Becker, E. D.; De Cabral Menezes, S. M.; Granger, P.; Hoffman, R. E.; Zilm, K. W. Further Conventions for NMR Shielding and Chemical Shifts (IUPAC Recommendations 2008). *Magn. Reson. Chem.* 2008, 46, 582–598.
- Mestrenova Research. MestReNova Manual © 2016. 2016, 238.
- [Link for NUTS/VNMR referencing through the Wisconsin Blog Spot](#)

### Appendix. Calculation of Shift changes relative to TMS:

- Use the SF command on the Bruker Spectrometers for your proton and heteronuclei of interest to determine the frequency of the nucleus on the specific spectrometer.
  - $\nu_{Hspec} = 399.9800000 \text{ MHz on the } 400NB$
  - $\nu_{Pspec} = 161.9148720 \text{ MHz on the } 400NB$
  - This value is set using dilute TMS in  $\text{CDCl}_3$  when the spectrometer is installed.
- Reference your spectrum using the Mestrenova referencing tool.
- Now to calculate the frequency of the standard X-nucleus reference,  $\nu_{X0}$ , needed to reference our heteronucleus.

$$\nu_{X0} = \nu_{TMS}^{obs} * \frac{\Xi_X}{100} \quad (1)$$

- Adding the difference, in Hertz, between the value of your unreferenced and referenced spectra to the  $\nu_{Xspec}$  value gives your referenced TMS value,  $\nu_{TMS}^{obs}$ .

Unreferenced  $CDCl_3$  frequency,  $\nu_{H0}$ , = 7.2842

Referenced  $CDCl_3$  frequency,  $\nu_{Hf}$ , = 7.2602

$$\Delta ref = 7.2842 - 7.2602 = 0.024 \text{ ppm or } 9.5995 \text{ Hz}$$

$$\nu_{TMS}^{obs} = \nu_{Hspec} + \Delta ref = 399980009.6 \text{ Hz or } 399.9800096 \text{ MHz}$$

- $\Xi_X$  is the frequency ratio, which relates the frequency of the  $^1H$  resonance of TMS to the frequency of a known X-atom resonance reference. Each nucleus and standard will have its own  $\Xi$  value, tabulated by IUPAC and included in MestreNova. In this case, we will be using  $\Xi$  value derived from  $H_3PO_4$  for our  $^{31}P$  spectrum.

Nucleus	$\Xi$ Value	Description
$^1H$	100.000000	Me4Si $CDCl_3$ , $\phi$ = 1%
$^2H$	15.350609	$(CD_3)_4Si$ neat
$^3H$	106.663974	Me $4Si-t1$
$^3He$	76.178976	He gas
$^6Li$	14.716086	$LiCl$ $D_2O$ , 9.7 mol/kg
$^7Li$	38.863797	$LiCl$ $D_2O$ , 9.7 mol/kg
$^9Be$	14.051813	$BeSO_4$ $D_2O$ , 0.43 mol/kg
$^{10}B$	10.743658	$BF_3 \cdot Et_2O$ $CDCl_3$ , $\phi$ = 15%
$^{11}B$	32.083974	$BF_3 \cdot Et_2O$ $CDCl_3$ , $\phi$ = 15%
$^{13}C$	<input type="checkbox"/> 25.144...	DSS methyl signal $D_2O$
$^{13}C$	<input checked="" type="checkbox"/> 25.145...	Me4Si $CDCl_3$ , $\phi$ = 1%
$^{14}N$	7.226317	MeNO2 neat/ $CDCl_3$
$^{15}N$	<input type="checkbox"/> 10.132...	$NH_3$ (liquid) external

Nucleus	$\Xi$ Value	Description
$^{19}F$	94.094011	$CCl_3F$
$^{21}Ne$	7.894296	Ne gas, 1.1 MPa
$^{23}Na$	26.451900	$NaCl$ $D_2O$ , 0.1 mol/dm $^3$
$^{25}Mg$	6.121635	$MgCl_2$ $D_2O$ , 11 mol/dm $^3$
$^{27}Al$	26.056859	$Al(NO_3)_3$ $D_2O$ , 1.1 mol/kg
$^{29}Si$	19.867187	Me4Si $CDCl_3$ , $\phi$ = 1%
$^{31}P$	<input type="checkbox"/> 40.480864	$(MeO)_3PO$ internal
$^{31}P$	<input checked="" type="checkbox"/> 40.480742	$H_3PO_4$ external

- Factoring these values  $\nu_X$  can be determined from eq 1:

$$\nu_{Xo} = \nu_{TMS}^{obs} * \frac{\Xi_X}{100}$$

$$\nu_{Xo} = 399980009.6 * \frac{40.480742}{100}$$

$$\nu_{Xo} = 1619148757 \text{ Hz or } 161.9148757 \text{ MHz}$$

- Next, the frequency of heteronuclei peaks can be determined using eq 2.

$$\delta_{x,ref}(ppm) = 10^6 (\Xi_{X,sample} - \Xi_{Xo}) / \Xi_{Xo} \quad (2)$$

- $\Xi_X$ , as the frequency ratio, is simply the quotient of an heteronuclei frequency and the referenced  $\nu_{TMS}^{obs}$ . Thus, the equation can be written:

$$\delta_{x,ref}(ppm) = \frac{10^6 \left( \frac{\nu_{X,sample}}{\nu_{TMS}^{obs}} - \frac{\nu_{Xo}}{\nu_{TMS}^{obs}} \right)}{\frac{\nu_{Xo}}{\nu_{TMS}^{obs}}}$$

- The  $\nu_{TMS}^{obs}$  factors and cancels, yielding eq 3.

$$\delta_{x,ref}(ppm) = \frac{10^6 (\nu_{X,sample} - \nu_{Xo})}{\nu_{Xo}} \quad (3)$$

- $\nu_{X,sample}$  is the unreferenced frequency of a peak in the heteronuclei spectrum in relation to the defined 0 ppm mark of the instrument,  $\nu_{Pspec}$ . In this example, we are looking at tris(o-tolyl)phosphine, with a peak at  $-29.6240$  ppm.

$$\begin{aligned}\nu_{X,sample} &= -29.6240 * \nu_{Pspec} * 10^{-6} + \nu_{Pspec} \\ \nu_{X,sample} &= 161910075.7 \text{ Hz or } 161.9100757 \text{ MHz}\end{aligned}$$

- Plugging these values into eq 3 yields our referenced chemical shift in ppm.

$$\delta_{x,ref}(ppm) = \frac{10^6(161.9100757 - 161.9148757)}{161.9148757}$$

$$\delta_{x,ref}(ppm) = -29.6456 \text{ ppm}$$