Tutorial:
Basic calibrations for solid state NMR experiments of membrane proteins

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Useful calibrations in protein solid state NMR

1. Referencing $^1$H, $^{15}$N, $^{13}$C and $^{31}$P

2. Basic VT calibration

3. Determination of frictional heating inside an MAS sample

4. Determination of power deposition on an NMR sample (static or MAS experiments)
1. Useful samples for referencing

- $^{15}$N ammonium sulfate, i.e., AMS (external reference). Set $^{15}$N resonance at 26.8 ppm.

- Adamantane, natural abundance (external reference). Set $^{13}$C high-frequency methylene resonance at 38.5 ppm.

- DSS in deuterated water. Set the DSS resonance to 0 ppm, use to verify $^1$H chemical shift of HDO (internal reference for water) and, indirectly, $^{13}$C chemical shifts ($v_0^C=0.251449530^* v_0^H$).

- $\text{H}_2\text{O}$ (internal reference) in fully hydrated samples. Set at 4.76 ppm at 298 K. Chemical shift decreases by ~0.1 ppm for 10 K increase.

- Pure $\text{H}_3\text{PO}_4$ (internal reference). Set isotropic $^{31}$P resonance at 0 ppm.

**NOTE: With external references, an effort should be made so that the reference volume and NMR tube/rotor are as close as possible to those of the actual sample, to avoid susceptibility effects.**
Further reading on NMR referencing

2. How to calibrate a variable temperature (VT) unit

Temperature is an important parameter for magnetic alignment, protein rotational diffusion and other dynamics. The variable temperature unit can be easily calibrated by $^1$H NMR.

- Prepare a sample of 100% ethylene glycol (273-416K) or 100% methanol (178-330K).
- Equilibrate the sample in the spectrometer (~10min)
- Record $^1$H NMR spectra at different VT settings.
- Use the $^1$H chemical shift difference $\Delta$ (ppm) between methylene/methyl and hydroxyl peaks to determine the actual temperature:

$$T \text{ (K)} = 466.5 - 102\times \Delta \quad \text{(ethylene glycol)}$$
$$T \text{ (K)} = 409 - 36.54\times \Delta - (21.85\times(\Delta^2)) \quad \text{(methanol)}$$

- Plot actual vs nominal (VT) temperatures to determine the correction for a specific VT unit.
Useful resources on temperature standards


• Online NMR temperature calculator:
  [http://www.spectroscopynow.com/userfiles/sepspec/file/specNOW/HTML%20files/NMR_temperature_measurement.htm](http://www.spectroscopynow.com/userfiles/sepspec/file/specNOW/HTML%20files/NMR_temperature_measurement.htm)
3. Frictional heating in hydrated MAS samples

- Knowledge of the actual sample temperature is important for rotationally aligned (RA) solid state NMR, and in any experiment where protein and/or lipid dynamics is important.
- Spinning liposome or other aqueous protein samples in an MAS experiment increases the inner sample temperature by frictional heating.
- Frictional heating depends on the MAS rate.
- Sample frictional heating can be dependent on chiller setting/air flow rate. A note should be made of these parameters.
- Using VT control, the sample temperature is well equilibrated in 10 minutes.
- The $^1$H H$_2$O resonance inside the sample can be monitored to verify the actual temperature changes in the sample.
Example: frictional heating in a 3.2 mm Bruker rotor (900MHz Low-E HCN probe, BTRC)

Gas flow: 1200 lph
\[ y = 1.1049x - 2.5399 \]
\[ R^2 = 0.99954 \]

Sample: Ethylene-glycol, verified on H\textsubscript{2}O resonance in biological sample
4. Determination of the power deposition in an NMR sample

- Power deposition during an NMR experiment can be significant in a hydrated biological sample, i.e., a “lossy” sample, and it is strongly probe/coil dependent.

- Lossy samples can be approximated by a 70 mM NaCl aqueous solution.

- $^1$H chemical shifts in Na$_5$[TmDOTP] are used for fast and precise measurements of RF heating (see Zuo et al in references).
How to measure RF heating

- Sample: 20 mM Na₅[TmDOTP] / 70 mM NaCl / D₂O

Na₅[TmDOTP]:
- Biocompatible
- Versatile: ¹H, ³¹P, ²³Na
- Paramagnetic: short recycle delay (d₁ < 500 ms)
- Can be also used to measure pH, ions, etc…
- Cost effective: <100$/gram from Macrocyclics (TX)

- Calibration curve: measure ¹H shift(s) of Na₅[TmDOTP] vs sample temperature in a VT-regulated 1-pulse experiment.
How to measure RF heating

A typical experiment to determine RF heating due to $^1$H irradiation:

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  RF on
  p
  90°
  Acquire
  d1
  n times = 8 min
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“Average” RF = $B_1^2 \times \text{Duty Factor} =$

\[= B_1^2 \times \left[ \text{time RF on} / \text{time RF off} \right]\]

where $B_1$ is in kHz
Further reading of RF heating and uses of $\text{Na}_5[\text{TmDOTP}]$


- Macrocyclics, TX: https://macrocyclics.com