Center for NMR Spectroscopy and Imaging of Proteins

UCSD

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

BTRC, 2014

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 ($\nu_0^{C}=0.251449530*\nu_0^{H}$).

- H_2O (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 H_3PO_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

- Wishart et al, J. Biomol. NMR (1995) 6, 135-140.
- Markley at al, Pure Appl. Chem. 70 (1998) 117.
- Earl & VanderHart, J. Magn. Reson. 48 (1982) 35–54.
- Morcombe & Zilm, Journal of Magnetic Resonance 162 (2003) 479–486.
- Harris et al, Solid State Nuclear Magnetic Resonance 33 (2008) 41–56.

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 Δ (ppm) between methylene/methyl and hydroxyl peaks to determine the actual temperature:

T (K)= 466.5 - 102*Δ (ethylene glycol)

T (K)=409 - 36.54*Δ - (21.85*(Δ^2)) (methanol)

- Plot actual vs nominal (VT) temperatures to determine the correction for a specific VT unit.

Useful resources on temperature standards

- Kaplan et al, *Anal. Chem.* **1975**, *47*, 1703
- Led & Petersen, *J. Magn. Reson.* **1978**, *32*, 1 - 17.
- Amman et al, *J. Magn. Reson.* **1982**, *46*, 319-321.

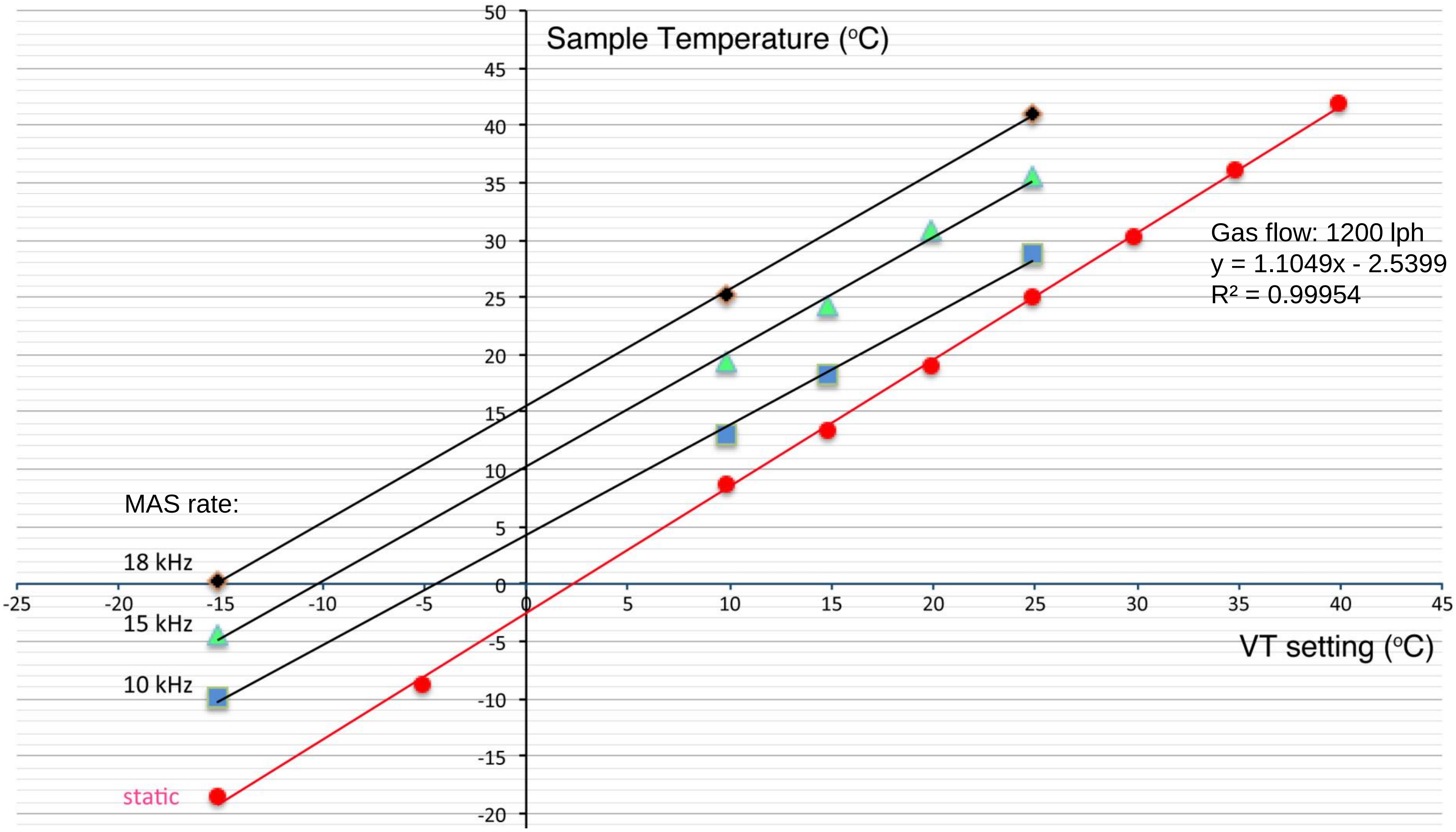
- Online NMR temperature calculator:

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_2O 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)



Sample: Ethylene-glycol, verified on H_2O resonance in biological sample

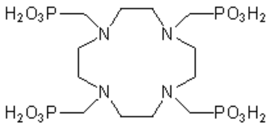
4. Determination of the power deposition in an NMR sample

- 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).

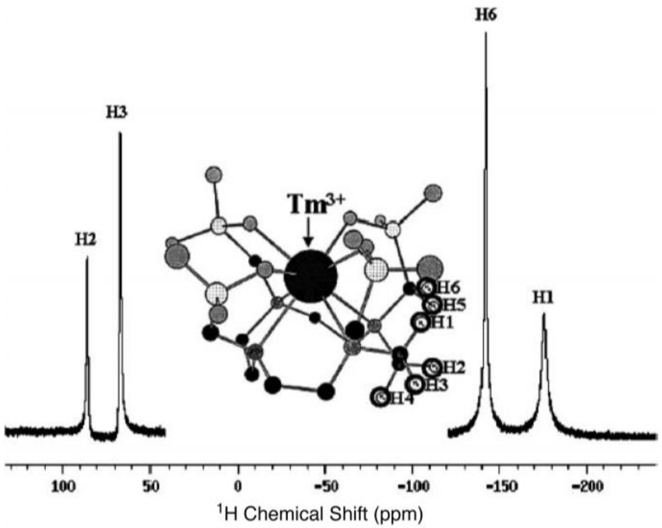
How to measure RF heating



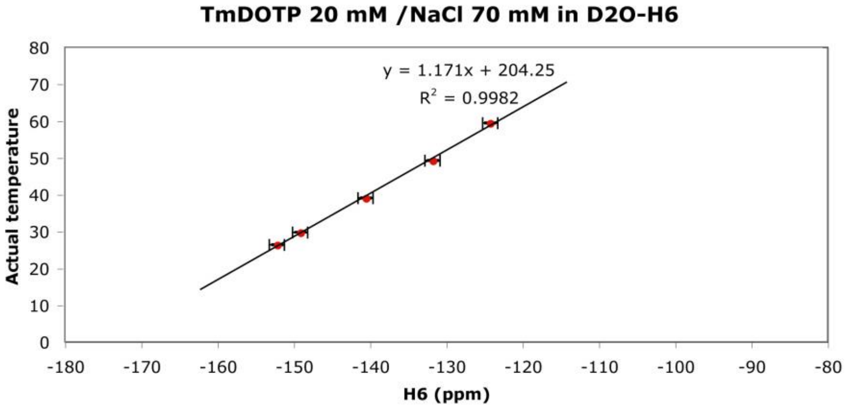
- Sample: 20 mM $Na_5[TmDOTP]$ / 70 mM NaCl / D_2O

$Na_5[TmDOTP]$:

- Biocompatible
- Versatile: ^1H, ^{31}P, ^{23}Na
- Paramagnetic: short recycle delay (d_1 < 500 ms)
- Can be also used to measure pH, ions, etc.…
- Cost effective: <100$/gram from Macrocyclics (TX)

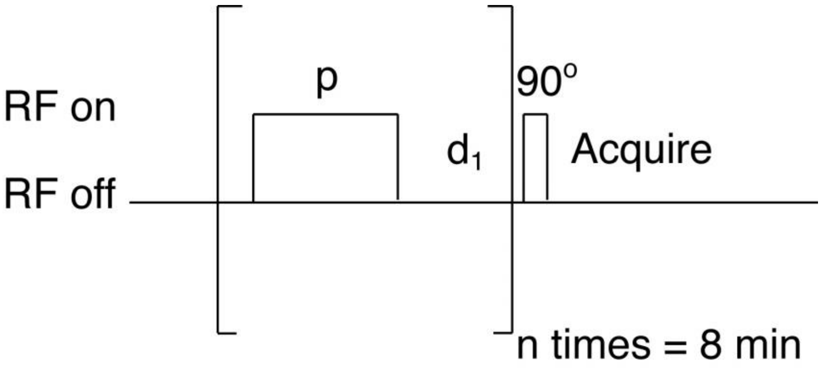


- Calibration curve: measure ^1H shift(s) of $Na_5[TmDOTP]$ vs sample temperature in a VT-regulated 1-pulse experiment.



How to measure RF heating

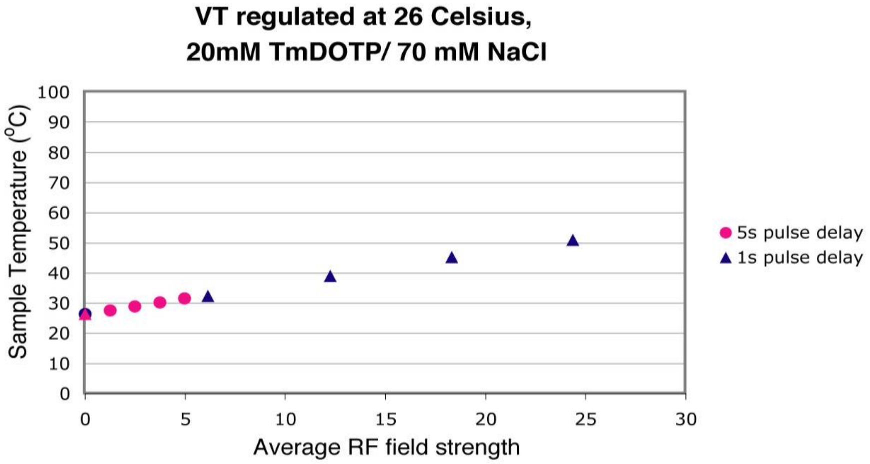
A typical experiment to determine RF heating due to ^{1}H irradiation:



"Average" RF = B_1^2 x Duty Factor =

= B_1^2 x [time RF on / time RF off]

where B_1 is in kHz



Further reading of RF heating and uses of Na_5[TmDOTP]

- Gadian & Robinson, J. Magn. Reson. 34 (1979) 449–455.
- Hoult & Lauterbur, J. Magn. Reson. 34 (1979) 425– 433.
- Kelly et al, J. Am. Chem. Soc. 124 (2004) 12013–12019.
- Li et al, Journal of Magnetic Resonance 180 (2006) 51–57
- Zuo et al, Magn Reson Med (1996) 36:955–959
- Zuo et al, J Magn Reson (1998) 133:53–60

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