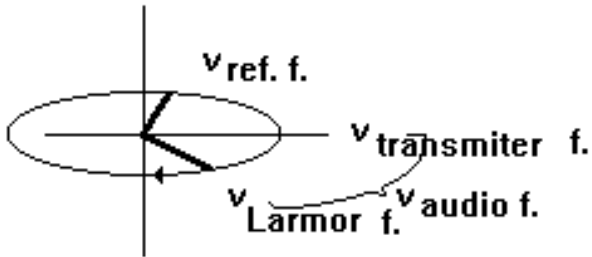# REFERENCING AND CROSS-REFERENCING

Obtaining a spectrum after DFT: - unit for the x axis is the frequency (ν),
                                    - unit for the y axis is intensity

$\nu_{\text{transmitter f.}}$ = rotating frame frequency (carrier frequ., transmitter frequ., zero offset frequ.)
$\nu_{\text{Larmor f.}}$ = Larmor frequency
$\nu_{\text{audio f.}}$ = audio frequency (size of the off-resonance, the off-set)

How do we get a spectrum? $\nu_0 = \omega_0/2\pi = \gamma B_0/2\pi$

        If γ is constant, characteristic for a nucleus type (e.g. ^{1}H or ^{13}C etc)
and B_0 is also a constant standing for the magnet, then ν_0 should be a single value.

In reality $\nu_0 = \omega_0/2\pi = (B_0 - \sigma B_0)\gamma/2\pi$ where σ is the screening or shielding constant.
In a different molecular environment the value of σ (a tensor [3x3 matrix]) is different.
        Memo: σ is anisotropic (σ_{xx}, σ_{xy}, σ_{xz}, σ_{yx}, σ_{yy}, σ_{yz}, σ_{zx}, σ_{zy}, σ_{zz})

    In liquids, molecule tumbles (all orientation is assumed) therefore we measure the time
    average over orientations: this is the isotropic value of $\sigma = (\sigma_{xx}, \sigma_{yy}, \sigma_{zz})/3$, the trace of the
    tensor.

Empirical effects influencing σ:

– **paramagnetic term** (electronic asymmetry generated by the electric field of neighboring nuclei) for ^1H no contribution, for other nuclei the dominant term. Therefore, ^1H spectrum smaller than that of other nuclei.

– **diamagnetic term** (electronic shielding of the nucleus) electron donating (or withdrawing) influence of neighboring functional groups

– **magnetic anisotropy term** (magnetic field generated due to anisotropy of groups) ring current shift originating from aromatic rings, carbonyl group anisotropy shift aldehyde protons

– **electric field term** (polar group induced electron distribution shift: electron drift, induced asymmetry) Important in ^{19}F shifts.

– **unpaired electron shift term** (large magnetic moment of unpaired electron) NMR of metals.

– **solvent effects**

      a) magnetic anisotropy [asymmetric benzene analogues]

      b) orientation effects [polar solute polar solvent]

      c) solvent solute interaction [H-bonding]
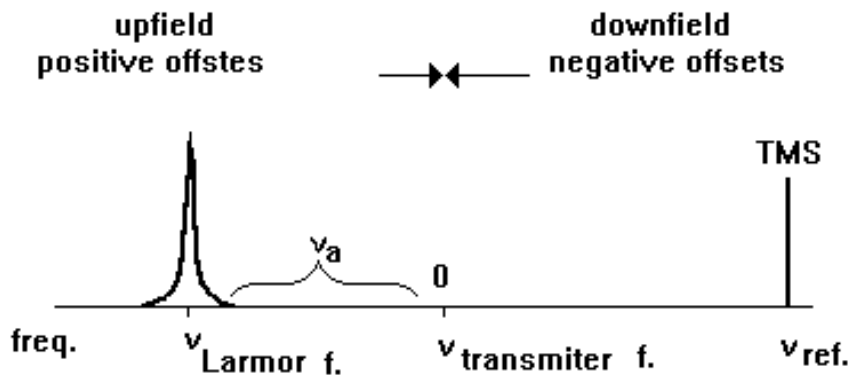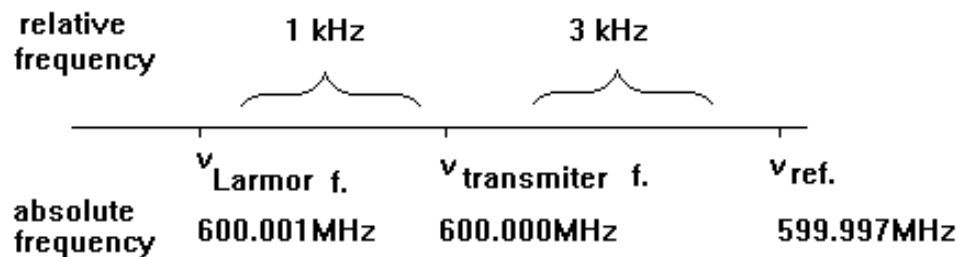
frequency scaling:

      problem: the absolute frequency value of a resonance depends on B_o
      solution: the relative frequency scale (δ) doesn't.

$$\delta = \frac{\nu - \nu_{ref}}{\nu_{ref}} * 10^6$$

$$\delta = \frac{600001000 - 599997000}{599997000} * 10^6 (ppm) = \frac{4000}{599.997}(ppm) = 6.6667(ppm)$$

what is a good reference?

- internal reference   - no interaction with the molecule of interest
                                                 - single resonance
                                                 - known T and pH dependence
                                                 - well resolved resonance from the resonances of the mol. of interest
                                                 -external reference

what to use as reference?

        - in organic solvent (e.g. $CHCl_3$)   -TMS [$(CH_3)_4Si$ as 0ppm] as ^1HNMR reference
        - in water   -TSP [$(CH_3)_3Si$-CD_2-CD_2-COO^-Na^+ as 0 ppm]
                                                        -DSS [as 0 ppm]

example how to use e.g. DSS as an external ref.
                            #1 measure a frequency of your mol.
                                  (e.g. the H_2O, the NH of a Trp, etc.)---> 600.001 MHz,
                            #2 take the sample out,
                            #3 assume that B_0 is not changing,
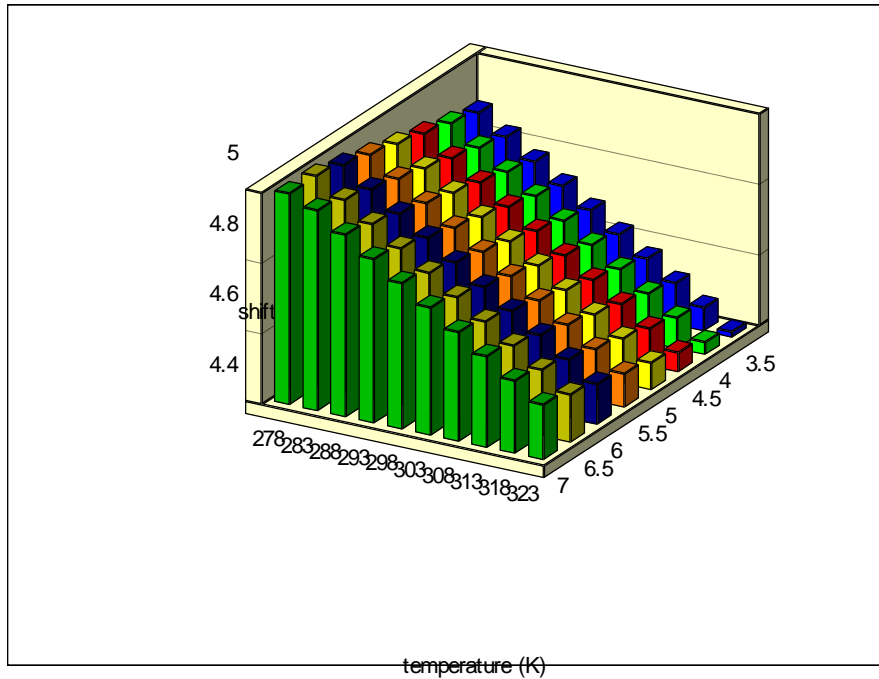   #4 put the DSS sample in,
                            #5 measure the DSS frequency ---> 599.997 MHz,
                            #6 reference the selected frequency as mentioned above.

use this data for cross referencing a NOESY, a TOCSY, a COSY recorded in a raw

repeat the referencing after adjusting   - the pH
                                            - the T
                                            - remeasuring the same sample at a different date.

Cross-referencing ^{13}C and ^{15}N via ^{1}H

problem: ^{13}C and ^{15}N are insensitive to direct observation
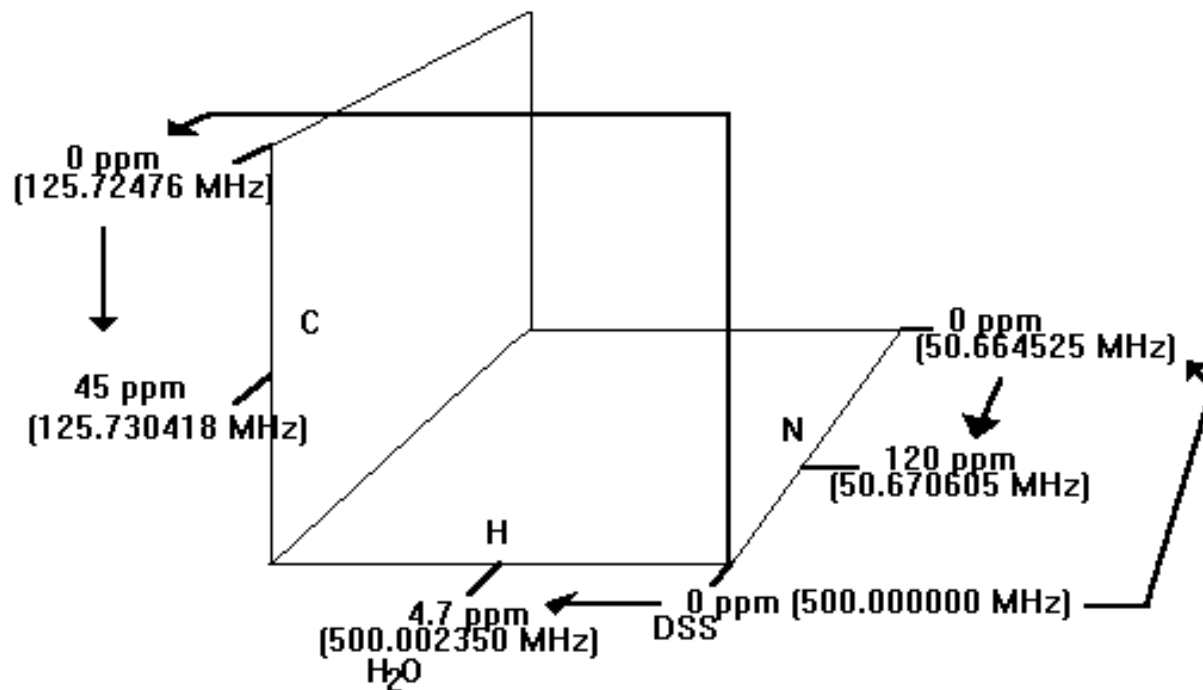*answer:* cross referencing

$$v_o^X = v_o^H \gamma_X / \gamma_H$$

where ν (s) are the frequency of the nuclei at 0 ppm

reference compound	γ_C/γ_H	γ_N/γ_H
DSS	0.25144952	0.10132905
TMS	0.25145002	0.10132914
TSP	0.25144954	0.10132900

example: if $\qquad$ $\upsilon_0{}^H = 500$ MHz (established by DSS)

$\qquad$ then $\qquad$ $\upsilon_0{}^N = 500 * 0.10132905$ MHz $= 50.664525$ MHz
$\qquad\qquad\qquad$ $\upsilon_0{}^C = 500 * 0.25144952$ MHz $= 125.72476$ MHz

For an ^{15}N HSQC centred on 120 ppm the required offset:

120 ppm $= (x - 50.664525)*10^6/50.664525 \;---> x = 50.664525\,(120 * 10^{-6} +1) =$

$= 50.664525 \; 1.000120 \text{ MHz} = 50.670605 \text{ MHz}$

For an ^{13}C HSQC centred on 45 ppm the required offset:

45 ppm $= (x - 125.72476)*10^6/125.72476 \;---> x = 125.72476\,(45 * 10^{-6} +1) =$

$= 125.72476 \; 1.000045 \text{ MHz} = 125.730418 \text{ MHz}$

The frequency of the water signal at pH $= 5.5$ and 25C is

4.7 ppm $= (x - 500)*10^6/500 \;---> x = 500\,(4.7 * 10^{-6} +1) =$

$= 500 \; 1.0000047 \text{ MHz} = 500.00235 \text{ MHz}$