

NMR Sample

Sample quantity

NMR has intrinsic-ally poor sensitivity

Concentration

Simple spectra (proton 1D) $\sim 50 \mu\text{M}$

Multidimensional spectra $\sim 0,5 \text{ mM}$

Sample tubes

5 mm, 550 μl

3 mm, 150 μl

Shigemi 5 mm, 250 μl

Solubility and stability

Aggregation, precipitation

NA better soluble than proteins

NMR measurements long

Run a control spectrum before and after a long experiment (1D, ^1H - ^{15}N HSQC for labeled proteins)

Molecular weight

Increasing molecular weight

Complexity of the spectrum – number of lines,
overlap

Increasing linewidths

Remedies

Isotope labeling

Multidimensional spectra

TROSY, MQ

NMR tube

Choice

Volume

Quality (geometry)

Cleaning

High quality tubes are expensive, not for single use!

Drying

No high temperatures above 50°C

Concentration, solvent exchange

Lyophilization

May harm samples (esp. proteins)

Ultrafiltration

Gel filtration

Ultracentrifugation

Isotope effect, H₂O vs D₂O

No D₂O is 100%

Viscosity

Melting/freezing point of D₂O is 3.8°C

pH

Glass electrode shows 0.4 units difference (7.0 in H₂O, 6.6 in D₂O), pH* value

Sample parameters

pH

Ionic strength

NMR probes (esp. cryoprobes) do not like high salt concentrations (above 100 mM)

Buffer type

Preferably buffer with no NMR signals

Concentration

Oligomerization, precipitation, high viscosity

Temperature

Sharper lines at higher temperatures, but not for exchangeable protons

Chemical shift reference

Internal

Add reference compound to the sample

External

Capillary within the sample tube

Direct

Reference compound measured together with the sample

Indirect

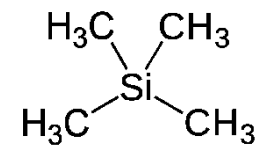
Reference compound measured separately or the chemical shift is calculated

Common reference compounds

Wishart et al. (1995) , J. Biomol. NMR 6, 135-140.

^1H

TMS 0.00 ppm, not soluble in water
TSP around 0, varies slightly with pH
DSS 0.00 ppm

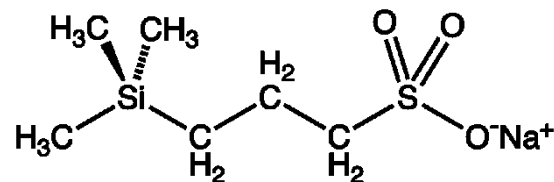


TMS

tetramethylsilane

^{13}C

TMS 1.7 ppm, not soluble in water
TSP around 0, varies slightly with pH
DSS 0.00



DSS

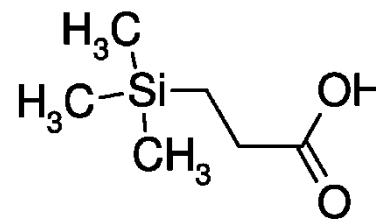
Sodium trimethylsilylpropanesulfonate
(dimethyl silapentane sodium sulfonate)

^{15}N

NH₃ 0.00 ppm, bio
CH₃NO₂ (neat) 381.7, chemistry

^{31}P

H₃PO₄



TSP

Trimethylsilylpropanoic acid

Contamination

- Paramagnetic contamination
 - Broadens the lines, destroys resolution
 - Cu ,Mn, Cr, Fe, Co
- Microbial contamination
 - Bacteria can eat-up your sample
 - Work clean, add sodium azide (toxic!), EDTA
 - Bacteria do not thrive in 100% D₂O