NMR Sample Preparation Guidelines

For all samples,
- all samples should be 550-600 μL in volume (e.g. 500-550 μL solution + 50 μL D_2O, see below)
- absolutely no bubbles, floating fibres or precipitate (i.e. spin down hard or filter)

If you are providing the sample ready-prepared in an NMR tube,
- use high quality NMR tubes (stores sell Wilmad 5mm 526-PP which are suitable), check for bends in tubes (by rolling on a flat surface) and that the base is exactly hemispherical (hold up to light)
- obtain extra long glass NMR pipettes (7") from Sigma in advance for filling the NMR tube (the facility staff can show you the appropriate technique to minimise bubbles if you are unsure)
- the cap alone provides a reasonable seal, but use parafilm and consider an N_2 or Ar purge if you wish to keep thiols in a reduced state
- label with the contents, your name and the date using a fine permanent marker on the glass near the cap, then cover the writing with a small piece of magic tape

For peptides, proteins, nucleic acids and carbohydrates,
- concentration >0.1 mM for 1D experiments
- concentration >0.5 mM for most 2D & 3D experiments (if this is not possible please discuss with the facility staff in advance)
- usually 90% H_2O/10% D_2O is best (7 - 10% D_2O is required for the field-frequency lock)
- use a buffer with no non-exchangeable H atoms if possible, e.g. phosphate. For e.g. TRIS, acetate or other additives such as DTT, EDTA, SDS, DPC, if the concentration exceeds 1 mM, depending on the experiment and labelling scheme it may be best to use deuterated versions of these compounds (please discuss this with the facility staff in advance)
- salt concentration (total ionic strength) ≤ 200 mM for room temperature probes, ≤ 100 mM for CryoProbes (none or as low as is practical gives best results)
- pH range for protein samples no greater than pH 8.0, 4.0-7.0 gives best results
- add 1-10 mM fresh DTT or TCEP as appropriate if you wish to keep thiols reduced

Optional extras,
- internal chemical shift reference e.g. 10 μM DSS or TSP
- azide (0.05%) prevents bacterial/fungal growths occurring in the tube over longer periods
- protease inhibitors can significantly extend the life of peptides/disordered proteins (important to discuss with staff first)

It's good practice to rinse new tubes with your solvent/buffer first. Tubes may be reused provided they are soaked in 50% nitric acid before copious rinsing with pure water before being allowed to dry. Best cleaning and drying is achieved by using a specially-designed NMR tube washer (available from Sigma).

Last modified 16/02/2011