Common Questions

1. What concentration should I use?

The table below is based on the *default settings* of the common protocols and gives the *minimum* recommended concentration when using those settings. You can use a lower concentration, but it will require more signal averaging (see question #3).

	Varian 300		Varian 500		Bruker 500	
Protocol	**time**	**conc.**	**time**	**conc.**	**time**	**conc.**
1D 1H	1 min	10 mM	1 min	2 mM	1 min	1 mM
gCOSY	6 min	40 mM	8 min	10 mM	8 min	5 mM
HSQC	8 min	60 mM	8 min	20 mM	6 min	10 mM
gHMBC	16 min	60 mM	16 min	20 mM	12 min	10 mM
1D 13C	32 min	60 mM	34 min	20 mM	9 min	10 mM
1D DEPT-135	9 min	60 mM	9 min	20 mM	3 min	10 mM
1D 31P	2 min	16 mM	2 min	4 mM	1 min	2 mM

2. How many *mg* of compound should I use?

If you have a target concentration in mind (e.g., from the table above), you can estimate the mass needed to prepare your NMR sample. First, use your molar mass (W, in g/mol) to estimate how many mg would produce 10 mM. Then scale this number of mg to produce the desire concentration. (For pure liquids with a density ~1 g/mol, this gives the # of µL to use; this is a good approx. for most non-halogenated organic molecules.)

$$\frac{W}{100} \rightarrow \#\, mg\ in\ 1\ mL \rightarrow 10\ mM$$

For example, the molar mass of caffeine is ~194 g/mol. Thus, ~2 mg will produce a solution of ~10 mM if ~1.0 mL of solvent is used. If you wanted a 20 mM sample, you would need ~4 mg. If you needed a 60 mM sample, you would need 12 mg, etc.

If you're being careful with your volumes, use 2 sig. figs. for your mass and the following will be more accurate estimates:

Varian (5 cm solvent height):

$$\frac{W}{100} \rightarrow \#\, mg\ in\ 0.67\ mL \rightarrow 15\ mM$$

Bruker (4 cm solvent height):

$$\frac{W}{100} \rightarrow \#\, mg\ in\ 0.55\ mL \rightarrow 18\ mM$$

Shigemi tubes (~300 µL is ideal):

$$\frac{W}{100} \rightarrow \#\, mg\ in\ 0.30\ mL \rightarrow 33\ mM$$

3. How long should I signal average?

If you have an estimate of your sample concentration, you can estimate the time that you will need using the data given in the table above. The key formula is

$$SNR \propto C\sqrt{t}$$

where C is the sample concentration and t is the acquisition time. This can be rearranged to predict the final time:

$$C_1\sqrt{t_1} = C_2\sqrt{t_2} \rightarrow t_2 = t_1\left(\frac{C_1}{C_2}\right)^2$$

For example, if your sample is ~5 mM, then we predict the following on the Varian 300 MHz instrument:

$$t_2 = 0.5\ hrs\left(\frac{60\ mM}{5\ mM}\right)^2 = 72\ hours$$

Whereas, on the Varian 500 MHz, we have

$$t_2 = 0.5\ hrs\left(\frac{20\ mM}{5\ mM}\right)^2 = 8\ hours$$

And, for the Bruker 500 MHz, we have

$$t_2 = 10\ min\left(\frac{10\ mM}{5\ mM}\right)^2 = 40\ min$$

4. How many t_1 increments should I choose?

Using a larger *number of t_1 increments* in 2D spectra will improve the resolution and reduce ringing artifacts along the F_1 axis. However, using too many will result in unnecessarily long acquisition times.

Note: your digital resolution in the F_1 axis is the spectral width (SW) divided by 2 and then divided by the # of t1 increments. For example, an HSQC with a ^{13}C SW of ~200 ppm and uses 96 t_1 increments will have a resolution of $\frac{\sim 200\ ppm}{2\times 96} \approx \frac{200\ ppm}{200} = \sim 1\ ppm$. For example, if you have ^{13}C signals that are closer than ~2 ppm, you would need to increase the # of t_1 increments in order to resolve them. Another approach is using a band-selective protocol over the crowded region, in which case the math is the same, but your SW is now much smaller.

# of t_1 increments for 2D protocols

	^1H-^1H (COSY, TOCSY, NOESY, etc.)			^1H-^{13}C (HSQC, HMBC, HSQC-TOXY, etc)		
Resolution	**Varian 300**	**Varian 500**	**Bruker 500**	**Varian 300**	**Varian 500**	**Bruker 500**
Low	128	256	256	64	64	96
Moderate	256	400	400	96	96	128
High	400	512	512	160	160	160

Note: Low = will work for very simple spectra; may get some ringing on tall signals.
Mod. = will work with typical spectra; these are the default values.
High = needed if you have crowded spectra in the F1 dimension.