1. Laboratory A measures the nmr spectrum of compound X at 400 MHz and reports that it has two classes of protons with chemical shifts of 1.43 ppm and 2.78 ppm with respect to the standard, TPA. Laboratory B measures the nmr spectrum of the same compound at 800 MHz and reports that it has two classes of protons with chemical shifts of 1.44 ppm and 2.79 ppm with respect to the standard TPB.

What is the chemical shift of TPB with respect to TPA? Why?

As chemical shift is independent of spectrometer frequency Lab B would also have measured 1.44 and 2.79 wrt to TPB at 400 MHz.

So TPB is –0.01 ppm wrt to TPA.

2. The following question covers material not explicitly presented this year-but which is present in the additional reading.

The compound CHCl₃ has a single proton with a chemical shift of 0.1 ppm. You conduct a COSY expt. in a 500 MHz spectrometer. After the first 90° pulse you wait a period of time \( t_1 \). Delta = 50 Hz; time for 1 rev in xy plane is 20 msec.

Where is the net magnetization of this proton (relative to an x, y, z coordinate system) immediately after the second 90° pulse for the following values of \( t_1 \):

a) \( t_1 = 0 \) msec. \( Mz = -1 \) (relative to value before first pulse)
b) \( t_1 = 10 \) msec \( Mz = +1 \)
c) \( t_1 = 15 \) msec \( Mz = 0 \) magnetization along x not affected
d) \( t_1 = 20 \) msec \( Mz = -1 \)
e) \( t_1 = 40 \) msec \( Mz = -1 \)
f) \( t_1 = 2.5 \) msec –tricky. My rotated about 1/8 rev = half way between y and \( -x \). The y component is flipped down so \( Mz = -0.5 \)

For each value of \( t_1 \) listed above what is the relative intensity of the absorption spectrum obtained from the Fourier transform of the Free Induction Decay recorded immediately following the second pulse (sketches or words)?

(a) None (no mag left in xy plane)
(b) ditto)
[c] max size but inverted-FID max size but starts by going negative.
[d] as [a]
[e] as [a]
3. You have recently inferred the amino acid sequence of a small protein by determining the sequence of its gene—the result is reliable. You now wish to determine the solution structure of this protein. Can this be accomplished using (a) COSY alone; (b) NOESY alone; or (c) COSY and NOESY together? **Explain.**

- Having the sequence does not allow you to skip any steps. You need both COSY and NOESY; the first so that all spots on the COSY-NOESY plot can be assigned to specific amino acids and the stereochemical data present in J values can be extracted. The NOESY so that distances can be determined and secondary structure assigned via repeats of characteristic distances.

In separate drawings sketch in a (relatively) quantitative manner the COSY and NOESY plots for the dipeptide ala-val. (Assume that these residues are present in positions other than the amino and carboxyl termini.) As an alternative to sketching you can construct tables which show the x,y coordinates of all the points you expect to find in the plots.

Ala has spots on the diag at NH, CH-alfa and CH3; valine will have spots at NH, CH-alfa, CH-beta and pair of methyls. The ala will show a crosspeaks from NH-CH-alfa and CH-alfa to Methyl. The valine will have NH-CH-alfa, CH-alfa-CH-beta and CH-beta to two methyls (and methyl to methyl?).

There should be a cross peak in the NOESY section from C-alfa of alanine to NH of valine.

4. **DBP (DNA Binding Protein)** has been extensively studied in your laboratory and its solution conformation determined using proton nmr. You now wish to establish whether or not the conformation of the protein changes when it bind to its cognate DNA—for this purpose you have a small oligo-deoxynucleotide which contains the necessary binding site. This DNA fragment is readily isolated from bacteria. Given that you have the complete COSY & NOESY maps of DNP what is the most straightforward way to use nmr to answer this question?

You want to avoid solving the structure of the protein-DNA complex so you isolate the DNA from bacteria grown in D2O. Then the DNA will not contribute to the proton nmr.

In the simplest case you just compare the COSY-NOESY map of the protein +/- DNA. If they are the same there has been no change in conformation, a very unlikely event.

5. In epr spectroscopy data is routinely recorded on both liquid and frozen samples. The epr parameters of vitamin B\textsubscript{12} (a cobalt compound, I = 7/2) are $g_x = 2.2$, $g_y = 2.2$, $g_z = 2.0$; $A_x = 0.000$, $A_y = 0.000$, $A_z = 0.003$ cm\textsuperscript{-1}. What is the derivative epr spectrum of this compound when recorded (i) in solution; (ii) at 77K. Assume the linewidth is sufficiently
narrow to allow any structure in the spectrum to be visible. Why are the two spectra of the same solution so different in shape?

A quantitative answer to this problem requires that you specify a spectrometer frequency. Let's use 9.2 GHz.

\[ g = 2; \quad H = 3283 \text{ gauss.} \]
\[ g = 2.2; \quad H = 2985 \text{ gauss.} \]
\[ Az = 0.003 \text{ cm}^{-1} \quad 90 \text{ MHz} = 32 \text{ gauss} \]

Frozen sample: Stick spectrum - a large peak at 2985 gauss from \( g_x \) and \( g_y \) of relative size 2. 8 peaks centered at 3283 gauss with a splitting of 32 gauss and relative size 0.125 (1/8 th of 1 g-value). The derivative will have the shape of the oblate ellipsoid (discus) with the high field trough split into 8.

Liquid sample: Average \( g = 2.133 \), average \( a = 0.001 \). An 8 line spectrum centered at 3078 gauss with the lines separated by 10.7 gauss. The derivative presentation has 8 derivative curves one at each of the field locations. They are equal height.

Explanation. In liquid samples of small molecules rapid tumbling averages the epr parameters and the spectra reflect these average values. In the frozen sample no averaging occurs and the intrinsic anisotropy is exhibited.
6. DMPO (5,5-dimethylpyrroline-N-oxide) is a small organic molecule used in biological research to detect the production of reactive oxygen species according to the equations:

\[
\text{DMPO} + \text{O}_2^- \rightarrow \text{DMPO-OOH}
\]

\[
\text{DMPO} + \text{OH}^\circ \rightarrow \text{DMPO-OH}
\]

where \text{O}_2^- and \text{OH}^\circ are the superoxide and hydroxyl radicals. The products of each reaction are also radicals and can be observed by epr. The epr parameters are \(a_N = 14\) gauss, \(a_H = 11\) gauss and \(a_H = 1\) gauss for DMPO-OOH and \(a_N = 14\) gauss and \(a_H = 14\) gauss for DMPO-OH.

When human neutrophils are stimulated by foreign bacteria in the presence of air and DMPO the epr spectrum shown in the following figure is obtained. What reactive form of oxygen is generated by the neutrophils?

It is necessary to compute the stick spectra for the two possibilities and compare with the data. Only DMPO-OH matches. It is not enough to compute one e.g. DMPO-OH and on seeing that it matches stop, because it might happen that the \(a\) values for DMPO-OOH conspire to yield the same spectrum. They do not but it needs to be documented.