BCMB/CHEM 8190
ANSWERS TO PROBLEM SET 7

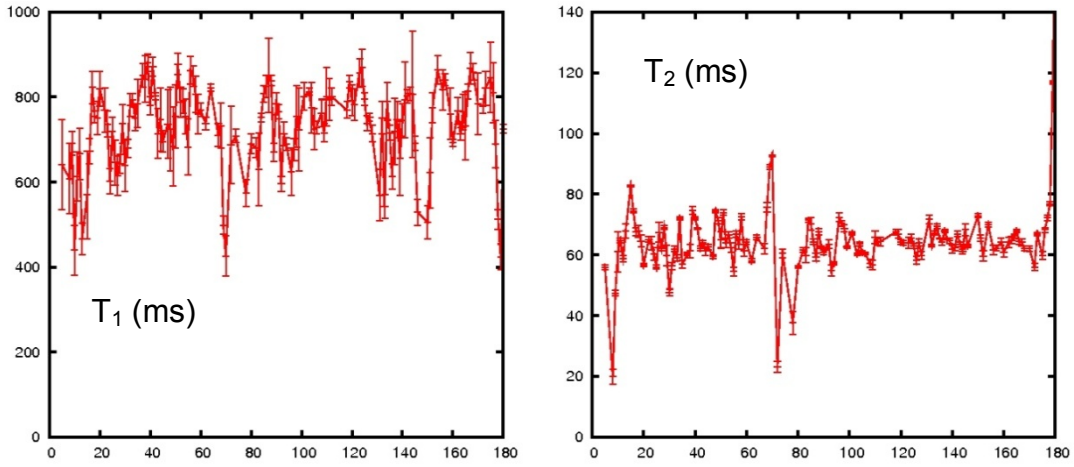
1) The splitting of a doublet for an amide proton-amide nitrogen pair in an HSQC spectrum that is proton coupled in the nitrogen dimension is measured as 94 Hz under isotropic conditions and 73 Hz under aligned conditions. What is the magnitude of the residual dipolar coupling for this pair? What is the sign of the coupling?

One-bond ^{1}H-^{15}N scalar couplings ($^{1}J_{HN}$) for amide groups in proteins are negative (γ for ^{1}H is positive, but γ for ^{15}N is negative). If the splitting is 21 Hz (94-73) and the measured scalar coupling (isotopic conditions) is -94 Hz, then the dipolar coupling is +21 Hz (-91+21 = -73).

2) Below we show some plots of values of ^{15}N T_1 and T_2 for many of the amide ^{15}N nuclei of a 21 kDa protein collected at 600 MHz (14.1 T magnet) and 25º C.

a) Using the simple formulas given in class estimate the correlation time (τ_m) for the protein using some of the data around residues 30-60.

b) Given that the dipolar interaction constant, *D*, for a H-N pair is 2.92×10^{9}, calculate the order parameter S^2 for these residues



a) We wrote a simple equation to predict τ_m from T_1 and T_2:

$$\frac{T_1}{T_2} \cong \frac{2}{3}\left(1+\omega_N^2\tau_m^2\right)$$

The frequency (in rad/s) for ^{15}N at 14.1 T (600 MHz for ^{1}H) is about 10 fold lower than for ^{1}H ($\gamma^{1}H/\gamma^{15}N \cong 10$), or about 60 MHz (then multiply by 2π for rad/s). From the T_1 and T_2 data for residues 30-60, a reasonable guess for average values of T_1 and T_2 are about 750 and 62 respectively. Thus:

$$\frac{T_1}{T_2} \cong \frac{2}{3}\left(1+\omega_N^2\tau_m^2\right) \quad \frac{750}{62} \cong \frac{2}{3}\left(1+\left(2\pi\times 60\times 10^6\right)^2\tau_m^2\right) \quad \sqrt{\frac{\left(\frac{750}{62}\frac{3}{2}-1\right)}{\left(2\pi\times 60\times 10^6\right)^2}} \cong \tau_m \cong 11 \text{ ns}$$

We also gave a "rule of thumb" in class that τ_m (in ns) ≅0.5 MW in kDa, so the answer is pretty close to that.

b) We derived simple expression for the spectral density, T_1, and T_2 assuming large molecules and small τ_e. For T_2 and the spectral density these are:

$$R_2 = 1/T_2 \cong (D/2)(4J(0)) \qquad J(\omega) = \frac{2}{5}\left(\frac{S^2\tau_m}{1+\omega^2\tau_m^2}\right) \cong \frac{2}{5}S^2\tau_m \text{ for } J(0) \text{ (zero frequency)}$$

We'll use T_2 from above, 62 ms (62 × 10^{-3} s). So, we can calculate S^2:

$$1/T_2 \cong \frac{1}{62\times 10^{-3}} \cong (D/2)(4J(0)) = \frac{4}{2}\times 2.92\times 10^9 \times \frac{2}{5}S^2 \times 11\times 10^{-9} = S^2 25.696 \quad S^2 = 0.63$$

3) The NMR spectrum of N,N-dimethylnitrosamine shows distinct *cis* and *trans* methyl signals with widths at half height of 1 Hz and a separation of 100 Hz. The inherent resolution (width at half height without contributions from exchange) is 0.5 Hz. Estimate the chemical exchange rate.

For a two-state, slow exchange first order exchange process:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

The lifetimes for states A and B (τ_A and τ_B, respectively) are:

$$\tau_A = 1/k_1 \qquad \tau_B = 1/k_{-1}$$

The exchange rate (k) and overall lifetime (τ) are related to τ_A, τ_B, k_1 and k_{-1} as:

$$k = 1/\tau = 1/\tau_A + 1/\tau_B = k_1 + k_{-1}$$

The linewidth at half height ($\Delta\nu_{1/2}$) includes the T_2 contribution and the contribution from exchange (exchange rate, lifetime):*

$$\Delta\nu = 1/(\pi T_2^*) + k/\pi = 1/(\pi T_2^*) + 1/(\pi\ \tau)$$

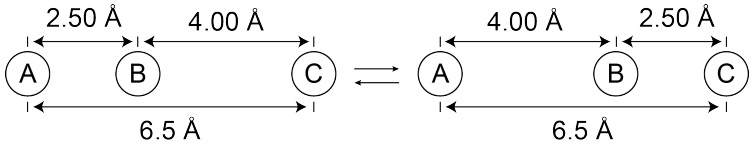
Here, the overall linewidth at half height is 1.0 Hz, with 0.5 Hz from T2. Thus the exchange rate and lifetime are:*

$$\Delta\nu = 1.0 = 0.5 + k/\pi \qquad k = (1.0-0.5)\pi = 1.57 s^{-1} \qquad \tau = 1/k = 0.64 s$$

4) Consider three ^{1}H atoms, A, B, and C in a molecule. Atoms A and C are constrained to be exactly 6.5 Å apart. "Medium" strength intensities of NOEs are observed between both A and B and C and B. Based on the strengths of these NOE intensities, we assign the minimum and maximum distances between A and B to be 2.0 Å and 3.3 Å, respectively. We assign the minimum and maximum distances between A and C to also be 2.0 Å and 3.3 Å.

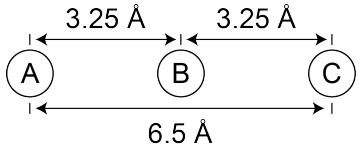
a) Estimate approximately where B is located relative to A and C.

b) Suppose the B atom really moves between two equally populated positions, one that is 2.5 Å from A and one that is 2.5 Å from C (see diagram). Assume the NOE observed is the $1/r^6$ average of the two positions. How does this compare to an NOE at a single position equidistant (3.25 Å) between A and C?



c) What is the distance between A and B when the NOE intensity for 100% occupancy is equal to the intensity calculated in 'b' for the average occupancy (50% 2.5 Å and 50% 4.0 Å)?

a) B must be located on a line connecting A and C



b) If we assume an NOE intensity of 1.0 for a distance of 3.25 Å, then the intensities for 2.5 Å and 4.0 Å, and the average, are:

$$\frac{3.25^6}{2.5^6} = \frac{I_{2.50}}{I_{3.25}} = \frac{I_{2.50}}{1.0} \qquad I_{2.5} = 4.83 \qquad \frac{3.25^6}{4.0^6} = \frac{I_{4.0}}{I_{3.25}} = \frac{I_{4.0}}{1.0} \qquad I_{4.0} = 0.288 \qquad I_{av} = \frac{4.83 + 0.288}{2} = 2.56$$

c) $$\frac{3.25^6}{(r)^6} = \frac{I_{av}}{I_{3.25}} = \frac{2.56}{1} \qquad r = 2.78 \ angstroms$$

5) In an SAR by NMR experiment two ligands, A and B, are found to perturb resonances in an HSQC experiment that belong to sites that are within 6 Å of one another. Ligand A has a dissociation constant of 2×10^{-4} M and ligand B has a dissociation constant of 5×10^{-6} M. If we successfully link these in such a way that their

individual binding geometries are not perturbed, and entropy effects are minimal, what would you expect to find for the dissociation constant of the linked ligand?

The dissociation constant is the product of the dissociation constants for the individual ligands:

$$K_d = \left(2 \times 10^{-4}\right) \times \left(5 \times 10^{-6}\right) = 1 \times 10^{-9} \text{ M or 1 nM}$$

6) We have a ligand of molecular weight 2000 that binds tightly, but with fast exchange, to a protein of molecular weight 300,000. At 800 MHz the ligand is giving negative NOEs by itself in solution. We would like to see transferred NOEs that are contaminated by no more than 20% by NOEs from the unbound state. What is the highest ligand to protein ratio that we could safely use?

The observed NOE is a weighted average of contributions from the free ligand and ligand bound to the protein (F_{free} and F_{bound} are the fractions of free and bound ligand):

$$NOE_{observed} = F_{free} \times NOE_{free} + F_{bound} \times NOE_{bound} \qquad F_{free} + F_{bound} = 1$$

If only 20% is to come from the free ligand, then:

$$\frac{F_{free}}{F_{bound}} = \frac{F_{free}}{1 - F_{free}} = 0.2$$

<u>However</u>, the rates of magnetization transfer and NOE buildup in the transferred NOE experiment, for large molecules, depends on the correlation time (τ_C), so are proportional to mass. In this case, the relative rate of build-up of NOEs will be 300,000/2000=150 (i.e. when bound to the protein, NOE buildup in the ligand is 150 times more efficient than in the free state……actually it is 302,000/2000=151, but we'll use 150).

$$\frac{F_{free}}{F_{bound} \times 150} = \frac{F_{free}}{\left(1 - F_{free}\right) \times 150} = 0.2 \qquad F_{free} = 30 - 30 \times F_{free} \qquad F_{free} = \frac{30}{31}$$

So, F_{free}=30/31, so F_{bound}=1/31, and the ratio of free ligand to bound is 30:1.

7) In an MRI experiment contrast agents are used to help resolve spatially distinct elements by selectively shortening T_1 or T_2 spin relaxation times for those elements. Images are usually displayed with regions giving the most signal as light areas and those giving the least signal as dark areas. Suppose we use an iron oxide nanoparticle as a contrast agent. Would you use a T_2 enhanced or T_1 enhanced sequence to acquire data? Would the areas affected by the nanoparticle appear as dark or light areas?

Iron oxide nano particles exert their greatest effect on T_2 – making it shorter. You would use a T_2 enhanced sequence. T_2 filters usually result in loss of signal from regions having the shortest T_2 – these regions would appear dark.