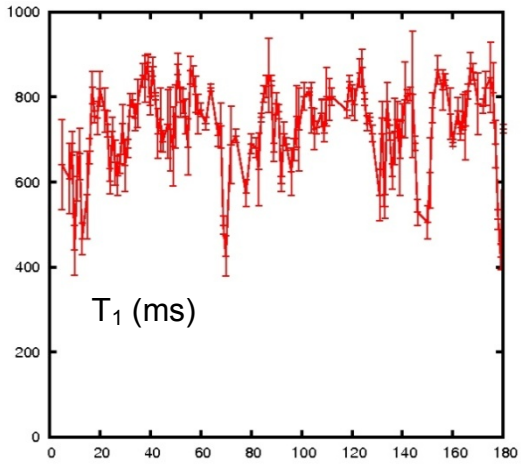
PROBLEM SET 7, BCMB/CHEM 8190

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?

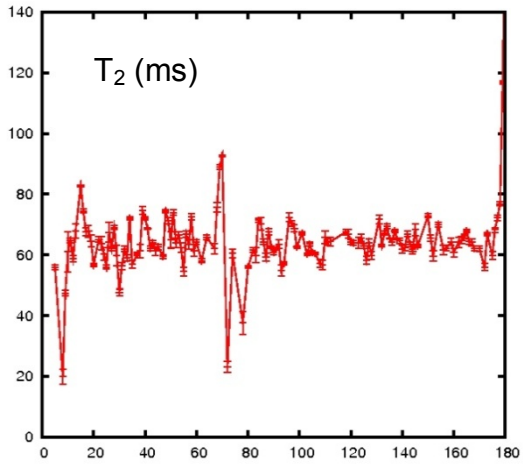
2) Below we show some plots of values of ^{15}N T_1 and T_2 for many of the amide ^{15}N nuclei of a 21 kDa protein collected at 600 MHz (14.1 T magnet) and 25º C.

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 x 10^9, calculate the order parameter S^2 for these residues



Sequence



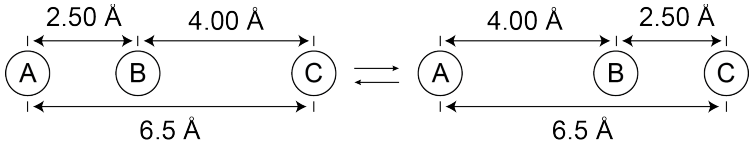
Sequence

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.

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)?

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?

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?

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?