07222016 HH/cgf

CPMG for ligand binding

Setup for CPMG experiments

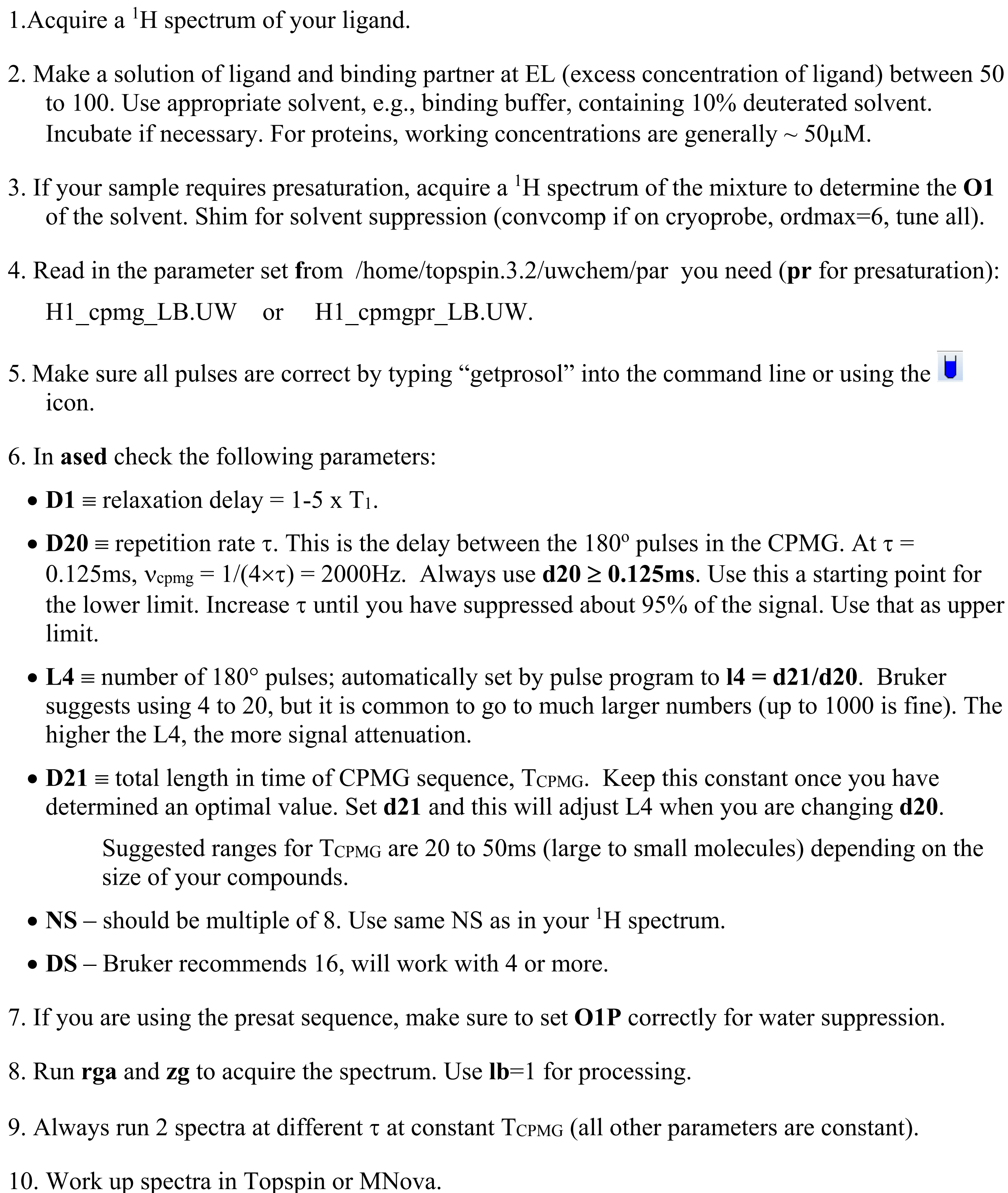
1.Acquire a ^{1}H spectrum of your ligand.

2. Make a solution of ligand and binding partner at EL (excess concentration of ligand) between 50 to 100. Use appropriate solvent, e.g., binding buffer, containing 10% deuterated solvent. Incubate if necessary. For proteins, working concentrations are generally ~ 50µM.

3. If your sample requires presaturation, acquire a ^{1}H spectrum of the mixture to determine the **O1** of the solvent. Shim for solvent suppression (convcomp if on cryoprobe, ordmax=6, tune all).

4. Read in the parameter set **f**rom /home/topspin.3.2/uwchem/par you need (**pr** for presaturation):

   H1_cpmg_LB.UW or H1_cpmgpr_LB.UW.

5. Make sure all pulses are correct by typing “getprosol” into the command line or using the  icon.

6. In **ased** check the following parameters:

- **D1** ≡ relaxation delay = 1-5 x T_1.
- **D20** ≡ repetition rate τ. This is the delay between the 180º pulses in the CPMG. At τ = 0.125ms, $\nu_{cpmg} = 1/(4\times\tau)$ = 2000Hz. Always use **d20 ≥ 0.125ms**. Use this a starting point for the lower limit. Increase τ until you have suppressed about 95% of the signal. Use that as upper limit.
- **L4** ≡ number of 180° pulses; automatically set by pulse program to **l4 = d21/d20**. Bruker suggests using 4 to 20, but it is common to go to much larger numbers (up to 1000 is fine). The higher the L4, the more signal attenuation.
- **D21** ≡ total length in time of CPMG sequence, T_{CPMG}. Keep this constant once you have determined an optimal value. Set **d21** and this will adjust L4 when you are changing **d20**.

  Suggested ranges for T_{CPMG} are 20 to 50ms (large to small molecules) depending on the size of your compounds.
- **NS** – should be multiple of 8. Use same NS as in your ^{1}H spectrum.
- **DS** – Bruker recommends 16, will work with 4 or more.

7. If you are using the presat sequence, make sure to set **O1P** correctly for water suppression.

8. Run **rga** and **zg** to acquire the spectrum. Use **lb**=1 for processing.

9. Always run 2 spectra at different τ at constant T_{CPMG} (all other parameters are constant).

10. Work up spectra in Topspin or MNova.

Determination of K_D

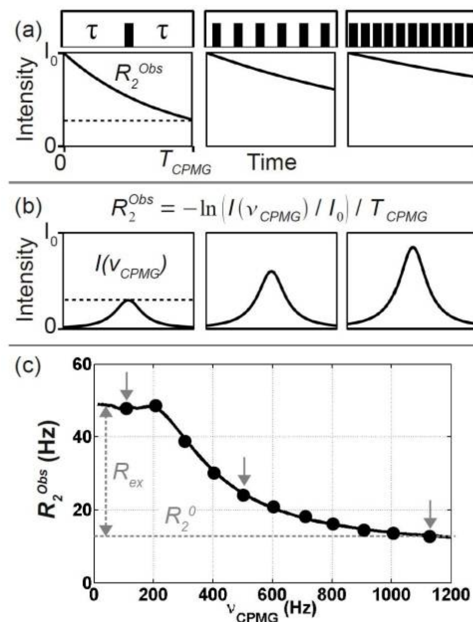
Only one sample is needed for this analysis.

1. Run cpmg experiments on sample with different delays τ between the 180° pulses (typically 10 to 20 spectra).
2. Make sure to cover the whole binding curve (0.125ms to 10ms [~2000Hz to 25Hz] – or whatever upper limit you determined for your system).
3. Work up spectra, carefully phase, correct the baseline and calibrate all spectra. Use lb=1Hz.
4. Calculate $\nu_{cpmg}=1/4\tau$
5. Calculate the relaxation $R_{2,eff}$ using the following equations.

$$R_{2,eff} = \frac{1}{T_2} = \frac{-1}{T_{cpmg}} \ln \frac{I}{I_0}$$

   Reminder: you cannot measure I_0. Extrapolate I_0. It is the largest value (from a really short τ or very long ν).

6. Plot $R_{2,eff}$ against ν_{cpmg}
7. Curve will also reveal R_{ex}.



In practice, a series of NMR spectra (for proteins, usually 2D ^1H-^{15}N or ^1H-^{13}C) are recorded containing a fixed relaxation time T_{CPMG} (~20-50 ms for large-small molecules), during which a variable number of spin-echos with different values of τ are applied sequentially (i.e., τ-180-τ, τ-180-τ, …) (figure 14). Each value of τcan alternatively be expressed as a CPMG frequency, $\nu_{CPMG} = 1 / (4\tau)$ that quantifies the rate of precession of magnetization about the axis of the applied RF pulse; typically 10-20 spectra are acquired using $\nu_{CPMG} \approx$ 50-1200 Hz. Importantly, the effective relaxation rate R_2^{Obs} is altered in a ν_{CPMG}-frequency-dependent manner such that significant refocusing is typically achieved when ν_{CPMG} exceeds half the exchange ratek_{ex}. The relationship between the *amount* of refocusing via R_2^{Obs} and the CPMG frequency is precisely the information used to fit the model of exchange.