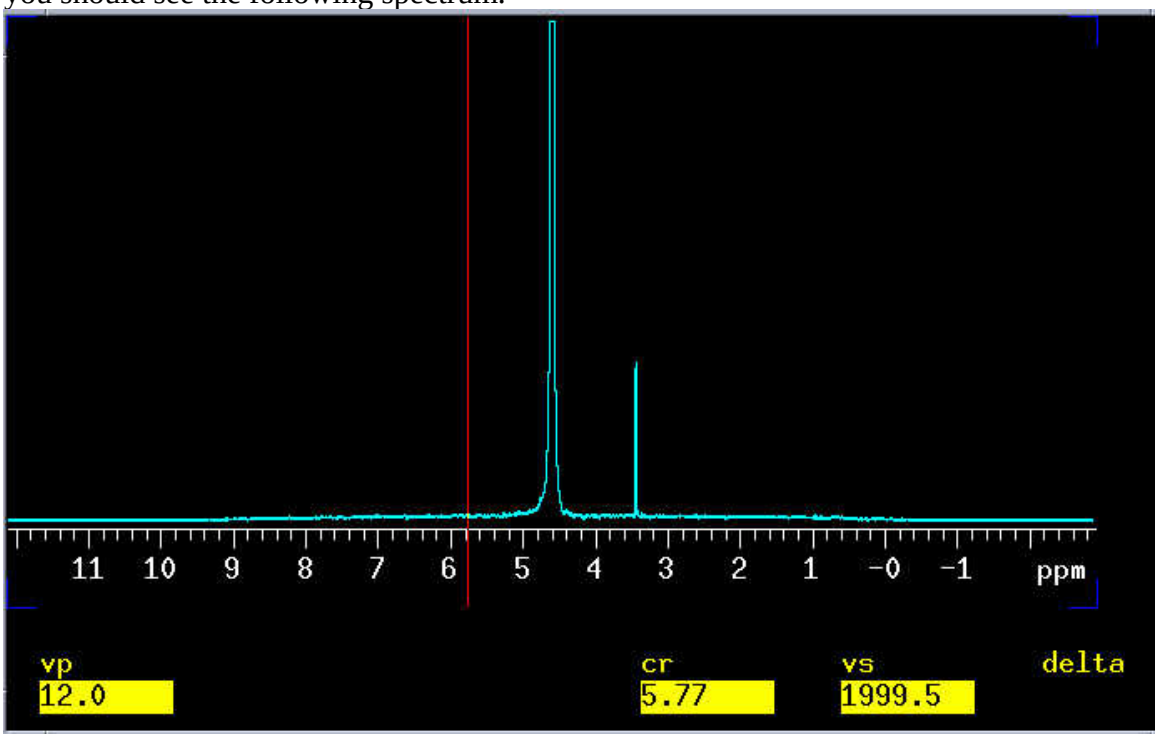
**Cobalt(II) protein NMR**

*This is a project currently being explored by myself between Chemistry and Agriculture The protein is a cobalt substituted peptide deformylase, an enzyme required for the growth of bacteria and weed. We are trying to find an effective inhibitor to this enzyme as antibiotics and herbicides.*

***Step 1. Record a normal 1D spectrum of Co_DEF on a 400 MHz machine.***

Setup the experiment using s2pul sequence;
**nt=1**, **tpwr=63**↵
**ga**↵
**aph**↵
Place the left cursor on the water peak, **nl** ↵
**movetof**↵
**ga**↵
**array**↵
**pw**↵ ***array pw90 and set pw=pw90.***
double check ***tof*** by typing **movetof**↵
**ga**↵
you should see the following spectrum.



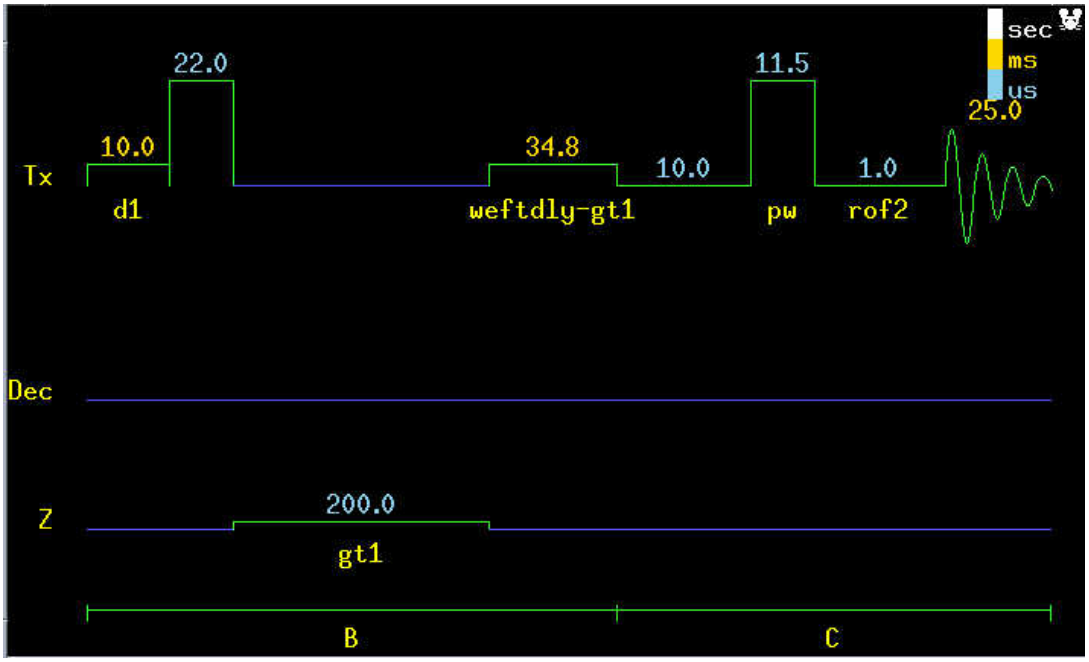
The parameters used for collecting this spectrum are listed as follows:

| ACQUISITION | | SAMPLE | | PROCESSING | | FLAGS | |
|---|---|---|---|---|---|---|---|
| sfrq | 399.730 | date | Jun 1 2004 | lb | not used | il | n |
| tn | H1 | solvent | D2O | sb | not used | in | n |
| at | 3.744 | file | exp | gf | not used | dp | y |
| np | 44932 | DECOUPLING | | awc | not used | hs | nn |
| sw | 6000.6 | dn | H1 | lsfid | not used | SPECIAL | |
| fb | 3000 | dof | 0 | phfid | not used | temp | not used |
| bs | 16 | dm | nnn | wtfile | | | |
| ss | 0 | dmm | c | proc | ft | | |
| tpwr | 60 | dmf | 11148 | fn | not used | | |
| pw | 16.2 | dpwr | 42 | math | f | | |
| p1 | 0 | | | | | | |
| d1 | 1.000 | | | werr | | | |
| d2 | 0 | | | wexp | | | |
| tof | -153.9 | | | wbs | | | |
| nt | 1 | | | wnt | | | |
| ct | 0 | | | | | | |

Please write down ***tof*** which is ***–153.9*** and ***pw*** which is ***16.2***.

## *Step2. Use WEFT sequence to run a protein NMR.*

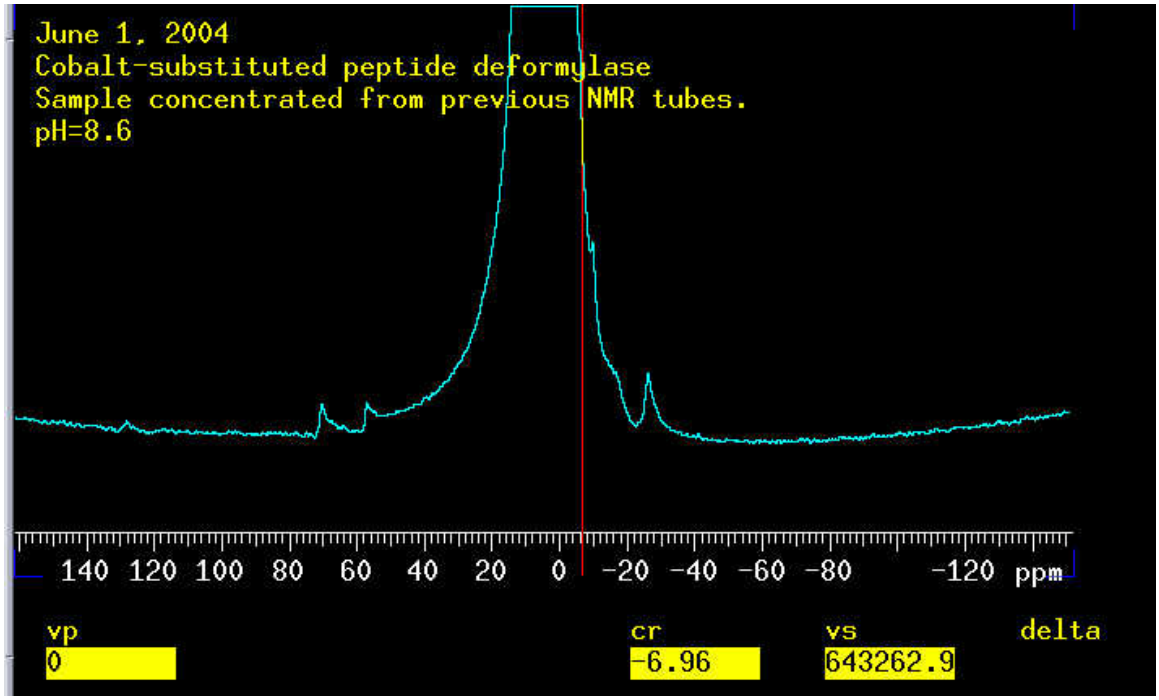
**jexp2↵**
we have a sequence called weftafhop, you are supposed to have one by contact you manager.
Load the original fid of a sample someone previously used, or the parameters.
Insert your sample(90%H2O and 10%D2O).
**dg↵**
**tpwr=63 nt=2048↵**
**pw90=11.5↵** ***( tpwr going up 60→63; so pw90 going down 16.2/1.414)***
***(If you want to know why, go to the pw90 calibration chapter)***
**pw=pw90↵**
**supfreq=tof↵**
**dps ↵**




**dg**↵

| ACQUISITION | | SAMPLE | | PROCESSING | | FLAGS | |
|---|---|---|---|---|---|---|---|
| sfrq | 399.730 | seqfil | weftafhop | lb | not used | il | n |
| tn | H1 | date | Jun 2 2004 | sb | not used | in | n |
| at | 0.025 | sample | WT FeSODred | gf | 0.003 | dp | y |
| np | 6250 | solvent | D2O | gfs | not used | pregrad | n |
| sw | 125000.0 | file | exp | awc | not used | prex | n |
| fb | 69000 | DECOUPLING | | lsfid | -2 | shape | n |
| bs | 16 | dn | H1 | phfid | not used | weftpls | y |
| ss | 256 | dof | 26321.5 | proc | lp | gradflg | y |
| tpwr | 63 | dm | nnn | fn | not used | satflg | y |
| pw | 11.5 | dmm | c | | | satpwr | 8 |
| p1 | 23.6 | dmf | 16949 | shape | n | gt0 | 0.001000 |
| d1 | 0.010 | dpwr | 8 | shapwr | 0 | gzlvl0 | 5000 |
| d2 | 0 | SPECIAL | | supfreq | -153.9 | pp | 8.05 |
| tof | -153.9 | temp | not used | pp | 8.05 | gt1 | 0.000200 |
| nt | 65536 | gain | 30 | pw90 | 11 | weftdly | 0.035000 |
| ct | 0 | | | | | gradly | 0.000100 |

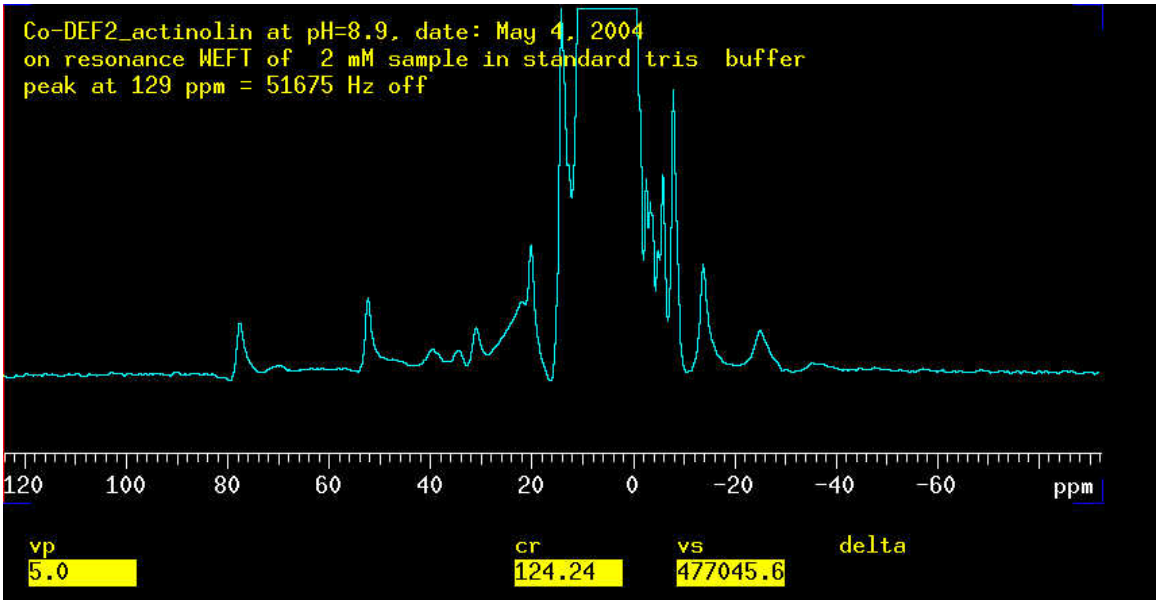
Things you need to take care of are:
***gain*** not exceed 30, ***satpwr*** smaller than 12.
**ga**↵




After the acquisition is done, you are supposed to have the above spectrum.

The inhibitor would react with the metal and stop the functioning of the protein. Here actinolin is used to chelate the cobalt atom. The same parameters are used for the NMR study.

A spectrum is shown on the next page.



***To do this job more informatively, you may do the following to optimize the parameters.***

1. **array supfreq (in the range ±15 is enough, because it is well set in the normal 1D)**
2. **array (rof2,alfa)**
3. **array (d1,weftdly)**

Keep in mind that **weftdly = at + d1**

You should manually phase the spectrum. Keep practicing and you will be able to phase it. Phasing error may occur.

To check the error, type **lp?↵;** if lp is far away from zero, then you need to correct it by typing **crof2↵**
**ga ↵**

You will get a better spectrum.

- **rof1**: receiver gating time proceding pulse 0-8190;
- **rof2**: receiver gating time following pulse 0-8190;
- **alfa**: After the final event in the pulse sequence, including any receiver gate times occurring following the pulse, acquisition occurs after a delay. This delay includes a fixed part, **alfa**, and a variable part, **1/(beta*fb).**

## How to array rof2 and alfa

**rof2? ↵ ( rof2=1)**
**alfa? ↵ (alfa=7)**
**array='(rof2,alfa)'**
**rof2=0,1,2,3,4,5,6,7,8**
**alfa=8,7,6,5,4,3,2,1,0**

***(remember: rof2 + alfa = 8 all the time)***

## How to array d1 and weftdly

**at? ↵** or **dg↵** to see what it is. ***at=0.025***
**array='(d1,weft)' ↵**
**d1=0.01,0.03,0.06,0.1↵**
**weft=0.035,0.055,0.085,0.125↵**

***(see that at + d1 =weftdly)***

Hint:

1. Do not miss the brackets, **"("** and **")"** when you type array='(xxx,yyy)' in this case, because if they are missing, you are arraying in 9 × 9 dimension which will run 81 jobs. You only need 9 jobs. Please type **da** to see the details in the text. **ds(1)** to display the first one, **ds(4)** to display the 4$^{th}$ spectrum. Compare all the spectra and select the best one. Use the best parameter to run protein NMR.
2. Use pure D2O as solvent to assign the peaks to the histidine moiety. To do this, you need to change the solvent and record another spectrum.