How to measure the following experiments on a GE

1. Preliminary setup

1.1. lock

-for proton set the transmitter frequency around 4.8 ppm	*(ppm=4.8)*
-for proton set transmitter power around 300	*(Tx power=300)*
-for a D_2O sample set transmitter power around 150	*(Tx power=150)*
-set the sweep width (sw) to a suitable value to have a relatively large and centered CW signal (e.g. set sw = 1000 Hz adjust the offset set sw = 250 Hz)	*(sw = 250 Hz)*
-set the lock gain to see a suitably intense CW signal	*(gain = 950)*
-set the lock offset to a value where the CW signal is centered	*(offset =-350)*
-set the lock phase to see a nice CW signal starting as a positive peak	*(phase = 120)*

1.2. shim

manual shimming: adjust according to the following scheme:

axial z1, z2, z1, z3, z1, (avoid if possible z4 and z5)

radial

back to axial shimming

compu shimming:

initial step	*50*
delay	*2*
ramp time	*2*
AQU	*1*
cycles	*1*

1.3. tuning the probe:

1.3.1. change the relevant cable for the „tune" cable

1.3.2. select the **setup/tune** command from the menu

1.3.3. optimize the signal (as intense and as sharp as possible) by changing the tune and the match

1.3.4. **quit** form the manual and reconnect the appropriate cable

1.3.5. go back or tune an other channel

2. Set and run a 1D ^{1}H experiment

2.1. load the *1Puls* pulse sequence *(seqload 1Puls)*

2.2. set F1 channel for proton *(f1 -a)*

nucleus	*none*
offset	*1.6Hz*
abs frequency	*500.1523816MHz*
rel frequency	*500.1523800MHz*

2.3. set
- -the sweep width equ. with 8000 Hz *(sw 8000)*
- -block size to 4k points *(cb 4k)*
- -pre acquisition delay (μs) *(de 72)*

2.4. adjust also

the gain (use xmt to avoid digitalization overflow)	*(gain 3)*
the number of scans	*(na = 8)*
relaxation delay (s)	*(rd = 3)*
the receiver phase (to avoid zero order phase correction)	*(rphase = 30)*
the number of dummy goes	*(dg = 8)*

2.5 run *(zg)*

2.6. process and display (Fourier trans., auto-phase, display) *(ft, aph, dis)*

3. Set for an irradiation (e.g. water suppression)

aim: to place the center of the peak (water peak) on the direct current (DC) signal to be able to use the F1 channel for presaturation.

3.1. Use a narrower spectral width (e.g. sw = 800 Hz) to have
a higher digital resolution for the same block size (e.g. cb = 2k).

3.2. Run the normal 1D experiment.
(*(zg, ft, aph, dis).*
The result is a broad water peak and a sharp direct current (DC) signal.

3.3. Adjust the f1 frequency to have the DC signal on the top (middle) of the H_2O signal
(f1+5h)

memo: if needed return to 3.2.

4. Measure the 90^o pulse

aim: to know the length of the time (µs) required at a given power level (e.g. 100% power level) to obtain the bulk magnetization to process completely in the [x, y] plane.

(e.g. seqload 1Puls)

4.1. Measure an approximately 45^o (or 60^o) 1D proton spectrum.
(pw 5, zg, ft, aph, dis)

4.2. Keep this phase and adjust the power (pw) to obtain now a 360^o 1D spec.
(pw 28.8, zg, ft, phase, dis)

4.3. Repeat until no signal intensity left.

4.4. Use the quarter of the final pw level (1/4*pw) as f190.

5. General settings for 2D experiments

memo: before running the 2D mode of a pulse run the appropriate 1D with a short t1dw (e.g. t1dw = 10µs).

before running count the phase cycling steps to set a proper number of scans na.

before running guess the value of T_2. From T_2 calculate t_{1max}.

From t_{1max} calculate the number of increments when setting up the acqmode command.

before running check the total time *(cg et)*

before running check the value of *t1dw* and make sure that it will reach t_{1max}.

6. Measure a NOESY

Estimate the mixing time.

7. Measure a TOCSY

One has to measure a high power (100% power level) and
a low power (e.g. 28% power level) 90^o.

The TOCSY mixing time (ms) is:

ilim * constant * low power 90^o (μs)

the constant is 4*2590/90

rule of thumb for TOCSY transfer in proteins

mixing time (ms) and expected transfer :

20 < < 30 from NH to H^α

30 < < 50 from NH to H^α and H^β

70< from NH to H^α, H^β ,H^γ and some of the H^δ

To have the affective value of the TOCSY mixing time (ms):

set ilim

run the TOCSY in a 1D mode

type *(cg pv)*

8. Measure a COSY

Run it with more t1 increments than the
appropriate NOESY and/or TOCSY *(e.g. acqmode 32 0 512 0))*

9. Measure a DQF-COSY

10. Measure a ^{1}H-^{15}N HSQC

10.1. calibrate f1 (^{1}H) as described previously (tune and the 90^{o} as described in 4.)
10.2 calibrate f3 (^{15}N) as below

10.2.1. tune the F3 channel (check the N filter box!)
10.2.2. calibrate the F3 90^{o} pulse length:
(use a suggested value or calibrate with a test sample using a suitable pulse sequence)

10.2.2.1. find a doublet (e.g. an NH proton signal which is a doublet because of the J^{NH} 91Hz)
10.2.2.2. set the power level and the f390 values e.g. (f390=5, and xlev=73)
10.2.2.3. run a 1D and phase it as an anti-phased doublet
10.2.2.4. keep the power level but alter the f390 values e.g. (f390=43, and xlev=73)
10.2.2.5. keep the values when the doublet vanishes such a value can be used also for N^{15} decoupling

(e.g. (f390=250, and xlev=53
use the (250*4)ms as value for f3 -g -> f3 -g 1000)

10.3. set (and adjust) the f3 frequency (f3 50.6856770 MHz)
10.4. run the HSQC (seqload glshsqc)
10.5. what are tau=2700, trim1=2000, trim2=1000 values?

11. Measure a ^{1}H-^{13}C HSQC

11.1. calibrate f1 (^{1}H) as described previously (tune and the 90^{o} as described in 4.)
11.2. calibrate f3 (^{13}C) as below
11.2.1. tune the F3 channel (check the C filter!)
11.2.2. calibrate the F3 90^{o} pulse length:
(use a suggested value or calibrate with a test sample (^{13}C glucose) using a suitable pulse sequence)
11.2.2.1. find the ^{1}H doublet (the H proton signal which is a doublet because of the $^{13}C1$: the presence of the J^{CH}).
11.2.2.2. set the power level and the f390 values e.g. (f390= 23.4, and xlev=0)
11.2.2.3. run a 1D and phase it as an anti-phased absorptive doublet
11.2.2.4. keep the f390
but alter the value of the power level e.g. (f390=23.4, and xlev=100)
11.2.2.5. keep the values when the doublet vanishes (zero intensity)
such a value can be used also for C^{13} decoupling
11.3. set (and adjust) the f3 frequency (f3 150.92600 MHz)
11.4. run the HSQC (seqload gls13ChsqcGrEn_co)
11.5. what are tau=1800, tau=900 values?