Diffusion measurement via the Gradient Echo

Knowledge of the diffusion coefficient, D, can tell you about the viscosity of a solution, or if the viscosity is known it can reveal the molecular weight or hydrodynamic radius of molecules in solution. If the solvent is water, it provides a built-in diffusion standard. Other solvents whose diffusion coefficient is known can equally well be used, although this example uses water.

This determination is broken into three portions: collecting the NMR data, -2- analyzing the results, -3- calculating the diffusion coefficient. The theory underlying equations used can be found in Stejskal and Tanner's original papers or in Pregosin, Martinez-Viviente and Kumar (2003) Dalton Trans. 2003, 4007-4014.

Collecting the data

In one workspace, collect a beautiful 1H1D and calibrate a pw90 for your sample. Also. it helps to know the T_1 approximately.

In a second workspace, load the parameter file wetPGEinvAFM.

As shown on **Figure 2**, this is set up to run the pulse sequence wetPGEinv_afm, which is a Pulsed Gradient Echo sequence incorporating the possibility of wet solvent suppression (see the handout on that topic).   As shown, the experiment is not implementing the wet solvent suppression capability [1] (In Acquire>Wet, WET is turned off).

The first pulse [2] is a 90° excitation pulse that brings all magnetization into the XY plane. A field gradient [3] is applied for a short time (2 ms here) causing spins at different heights in the NMR tube to get out of phase from one another (dephasing). Next, there are a pair of 90° pulses applied along the same axis [4]. These can be thought of as two halves of a 180° pulse, for this experiment. Thus, the experiment can be regarded as a spin echo with gradients in the two flanking delays [3] and [5]. However in between the two central pulses, a delay is allowed during which molecules will diffuse within the sample tube [6]. Small molecules that diffuse fast may move substantially during this time and therefore end up at a different height in the sample. The second half-180° returns magnetization to the XY plane where the second gradient [5] can refocus the dephasing produced by the first gradient. However refocussing will only perfect for molecules that are at the same position in the tube for both gradients, because only they will experience the same field strength. The further a molecule diffuses (on average) the less magnetization is refocussed. Thus, small molecules with high diffusion coefficients will loose more intensity than larger molecules with low diffusion coefficients. Intensity can also be lost due to relaxation. In order to distinguish between losses to relaxation *vs.* losses due to diffusion, we will repeat the experiment with a series of different gradient pulse strengths. Losses due to relaxation will be constant whereas losses due to diffusion will increase in proportion to the square of the gradient strength.

In Acquire>Acquisition (**Figure 3**), enter your values for <u>sw</u> [1], <u>at</u> [2], <u>d1</u> [3] <u>pw90</u> [4] and tpwr [5] based on your beautiful calibrated 1H1D. (To enter , <u>tof</u> you will have to go to Acquire>Channels.) Note that this pulse sequence resembles the simple 1D pulse sequence in that the excitation pulse is not necessarily set to 90°. Check the value of the 'first pulse' [6] and change it to your <u>pw90</u> value. (You can also do this by typing **pw=pw90** to make all the pulses 90° pulses. Check the 'Sequence' [7] display and confirm that all the pulse lengths [8] have the value of your <u>pw90</u>. Also recall that the relaxation delay <u>d1</u> should be appropriate for your sample, (eg. d1 ≈ 1.3 * T_1).

The diffusion delay d5 [9] should be shorter than T_1 , but will depend primarily on the diffusion coefficient. We will choose it based on a test run (more below).

First set gzlvl=0 by typing in the command line [1] (**Figure 4**) . We do not have a GUI set up for the PGE-specific parameters yet. To confirm that your value has been accepted, click on 'Sequence' again and see that the height of the gradient pulses is now zero [2]. Also, view the PGE parameter set by going to the Acquire>Overview panel [3], [4]. (I had d5=.5 and gradient pulse durations of 0.1 seconds when this screen shot was taken.)

Collect a spectrum and correct the phase. Set 'Future Actions' for when the experiment finishes to 'wft dc ds' to weight and Fourier transform (=wft), level the spectrum (drift correct=dc) and display (=ds).

Now activate the gradient pulses again by typing in an array of values for gzlvl (**Figure 5**). Because we will want the values of $gzlvl^2$ to be evenly spaced, we will enter our list of gzlvl values by hand instead of using the 'Arrays' button. Type (for example) **gzlvl=100, 600, 1100, 1500, 1800, 2000** [1]. You will not be allowed to use a value larger than 2047, in order safeguard the instrument (2047 is the maximum on GORT, large values apply for T2J and T1J, which have older hardware.)

Click 'Show Time' [2], choose a number of scans that will give you signal-to-noise of at least 20:1 (eg. **nt=8**). Acquire [3].

Fourier transform all your spectra and display them side-by-side either using the buttons in the 'Arrayed Spectra' panel on the left-hand-side [4] and arraying horizontally [5], or by typing **dssh** (display spectra stacked horizontally). You see that the stronger gradients result in greater signal loss for the same amount of diffusion in all cases (all cases use the same value of d5.)

<u>Analyzing the results</u>

The intensity of the signal, I, depends on the gradient strength G and diffusion constant D as follows,

$$\ln\left(\frac{I}{I_o}\right) = -(\gamma\delta)^2 G^2\left(\Delta - \frac{\delta}{3}\right)D$$

where I_o is the signal intensity obtained from the same experiment when the gradient strength is zero. γ is the gyromagnetic ratio of the nucleus being observed (for ^{1}H it is 4258 Hz/G) and δ and Δ are time intervals in the pulse sequence (**Page 6**).

δ = gt

Δ = gt + d2 + 2pw+ d5 + d0

Rearranging the equation, we get:

$\ln(I) - \ln(I_o) = -(\gamma\delta)^2 G^2(\Delta - \delta/3)D = -(\gamma\delta)^2(\Delta - \delta/3) D G^2$

$\ln(I) = \ln(I_o) - (\gamma\delta)^2(\Delta - \delta/3) G^2 D$

Predicts that $\ln[I]$ vs. G^2 should be a straight line with a slope of $-(\gamma\delta)^2(\Delta - \delta/3)$ D.

We don't yet know what our G values are (see below), but we know they are proportional to gzlvl, so we propose: G=grad_cal x gzlvl, where grad_cal is the conversion factor from Varian units to G/cm gradient strengths. Taking that into account we predict that a plot of the natural log of signal intensity, $\ln[I]$ vs. $gzlvl^2$ should be a straight line with a slope of $-(\gamma\delta)^2(\Delta - \delta/3)$ $grad\_cal^2$ D.

For the example of water, an actual plot of $\ln(amplitude)$ vs. $gzlvl^2$ is shown in **Figure 7**. This shows that we do indeed get a straight line with a negative slope.

To execute this analysis on your own data you will need to extract the peak intensities foe each peak in each spectrum. Once the data collection is complete display the first spectrum by typing the **ds(1)** command. (**Figure 8**). Go to the Process>Integration panel [1], activate integral display [2]. Select 'clear integrals' [3] and then 'interactive Resets' [4]. Now place resets on either side of each peak by clicking with the left-mouse beginning on the left side of each signal.

Now type **bc** to use the regions not selected as a basis for baseline correction. To apply the same baseline correction to each of the other spectra in your array, type **ds(2) bc** then **ds(3) bc** , then **ds(4) bc** , etc until all the spectra have corrected baselines [5].

To see all your spectra one above another, type dssa (**Figure 9**)

Now we want to extract peak amplitudes for each peak in each spectrum. Display the first spectrum by typing the **ds(1).** command results. In Process>Display (**Figure 10**), select the threshold icon [1] . Then click on 'find peaks' [2] to show them with their ppms. **Figure 11** shows the result . Make a note of which peaks are of interest.

To get amplitudes for these, go to Process>Cursors / Line Lists (**Figure 12**). Click on Display Line List [1], check that you see the peaks you saw in findpeaks. Then click on Find Peaks in Array [2].

Scroll down with the right-hand scroll bar [3] to reveal a horizontal bottom-of-window scroll bar [4]. Scroll that all the way to the right to reveal two vertical scroll bars [5], the inner one of which allows you to see the peak amplitudes for each of your lines in each spectrum.

Use the mouse to select all the data, click Ctrl 'c' to copy it . (**Figure 13**) Under Applications>Office [1] go to OpenOffice.org > Writer

In the window that opens, click Ctrl 'v' to paste in all your data. Save the text file with a useful name in a useful location, and close it. Transfer this file to your own computer and reopen it in a spreadsheet package such as excel. Keep the data for a given peak together [2], and copy it to a column in your spread sheet.

Do this for each peak that interests you so that you have one column for each peak [2] next to columns for gzlvl an gzlvl^2 values used in each spectrum [1] (**Figure 14**).

You will then need corresponding columns for the natural logs of the peak amplitudes [3]. Plot these vs. the square of gzlvl (I used gzlvl/100 all squared in order to not have huge numbers) [4].

If your sample employs water as the solvent, you can easily use internal water as your calibration because its diffusion coefficient is known (below). If not, we assume that you are using a previously-determined value for the calibration coefficient grad_cal. Alternately, you can look up the diffusion coefficient of your solvent at your temperature, and use it in the same manner as water is used below.

Now generate an XY chart or plot of the ln (amplitudes) vs. gzlvl^2 values (**Figure 15**), and obtain the slopes of all the lines. If the lines are not genuine straight lines, then your data do not fit the model. Re-examine your analysis, your sample, your pulse sequence etc, but do not proceed unless the lines really are straight lines.

Using your values of $-(\gamma\delta)^2(\Delta- \delta/3)$ grad_cal^2 (below or prior calibration of grad_cal), use the slopes to calculate D for each of the molecules in the sample, based on the slopes obtained from signals from each molecule (**Figure 16**). (Recall that slope divided by $- (\gamma\delta)^2(\Delta- \delta/3)$ grad_cal^2 = D. In the example sample there were two species dissolved in water. One was found to have a diffusion constant around 8 x 10^{-10} M^{2}s^{-1}, and a larger one with a slightly slower diffusion rate with D $\approx$ 5 x 10^{-10} M^{2}s^{-1}.

Short cut if your solvent is water

For samples dissolved in water there is no need to actually determine the individual constants, since water can be an internal standard and the entire $-(\gamma\delta)^2(\Delta-\delta/3)$ term is constant. Recall that slope $= -(\gamma\delta)^2(\Delta-\delta/3)$ grad_cal^2 x D and for water I had a slope $= .0114$ x $10^{-4} = -(\gamma\delta)^2(\Delta-\delta/3)$ grad_cal^2 x 1.8 x 10^{-9} M^{2}s^{-1} . Therefore, $(\gamma\delta)^2(\Delta-\delta/3)$ grad_cal$^2 = 633$ in this case, for the delays I used.

However, for samples dissolved in water, the challenge is to combine the diffusion measurement with water-supression.

Go back to your beautiful 1D, which will be dominated by the signal of water. In that workspace, you can make a shaped pulse for water suppression (see the handout on wet solvent suppression for more detail).

Make a shaped puse using 'Edit > New pulse' (**Figure 17**).

In the Acquire>Wet panel, check the 'WET' box (Figure 2) . The pulse sequence now displays the WET pre-sequence (**Figure 18**).

Optimize the WET pulse power if needed, with <u>gzlvl</u>=0. Then set up your <u>gzlvl</u> array as above.

Gradient calibration

In order to obtain numerical values for the diffusion rate of your molecules, you need to know how strong the gradients are. In other words, we need to know what gradient strengths correspond to the values of gzlvl. We determine the conversion factor by performing our experiment on a molecule whose diffusion coefficient is known. In this case, water was used, because at 25 °C, we know that the diffusion coefficient of water is D=1.8 x 10^{-9} M^2/s.

We will use the slope obtained for water at 25 °C to calculate a conversion factor that multiplies our gzlvl values to give G.

The slope $= -.0114$ x 10^{-4} (G/cm)$^{-2} = -(\gamma\delta)^2(\Delta-\delta/3)$ D grad_cal^2 and ln(I$_o$) = 4.65

D=1.8 x 10^{-9} M^2/s

In this case d0= 50 us, gt = 2 ms, d2 = 50 us, pw=14.75 us and d5 = 200 ms.

Thus δ= .002 s and Δ= (.002 + .00005 + .0000295 + .2 + .00005) s = .2021295 s

$\Delta-\delta/3$ = .201463 s

slope = (4258 Hz/G * .002 s)^2 (.201463 s) 1.8 x 10^{-9} M^2 s^{-1} grad_cal^2 = 0.0114 x 10^{-4} (taking into account my having divided gzlvl by 100 before squaring.

72.5 G^{-2} x .201463 s x 1.8 x 10^{-9} M^2 s^{-1} grad_cal^2 = 0.0114 x 10^{-4}

2.63 G^{-2} M^2 grad_cal^2 = 114

grad_cal^2 = 43.3 G^2 /M^2　　　grad_cal = 6.6 G/M = .066 G/cm

Thus, our top value for gzlvl of 2000 corresponds to 132 G/cm or 13,200 G/m gradient **sounds low**
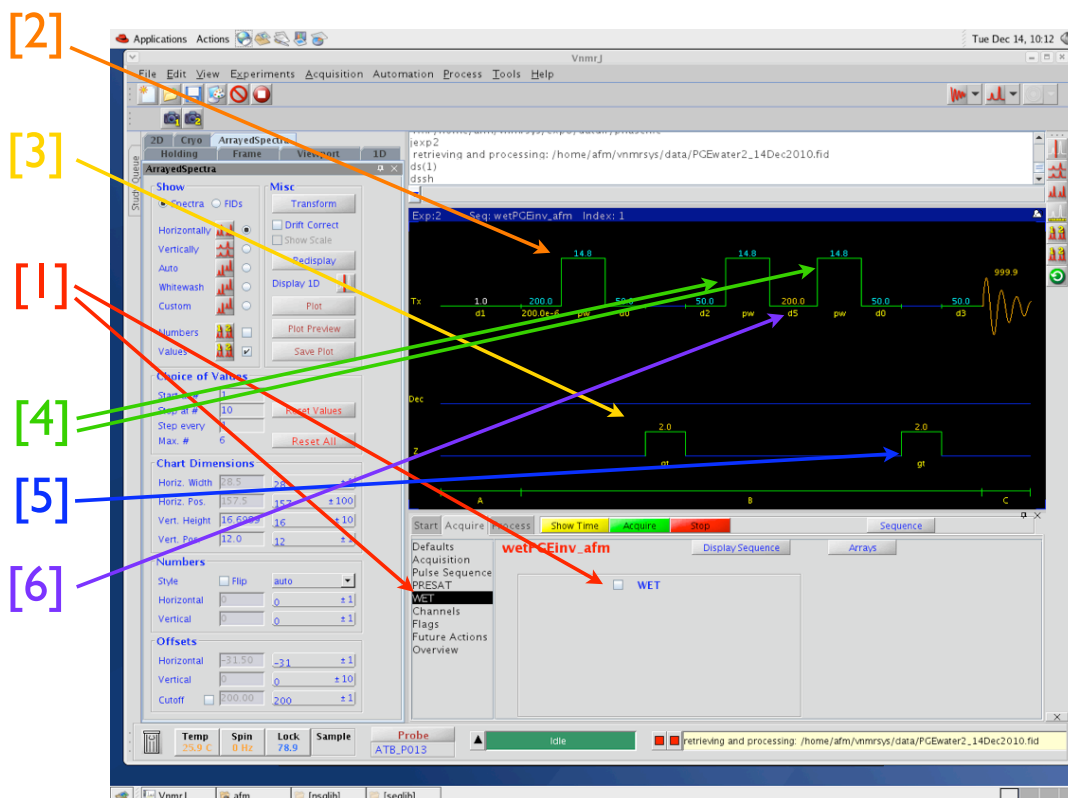
PGE:
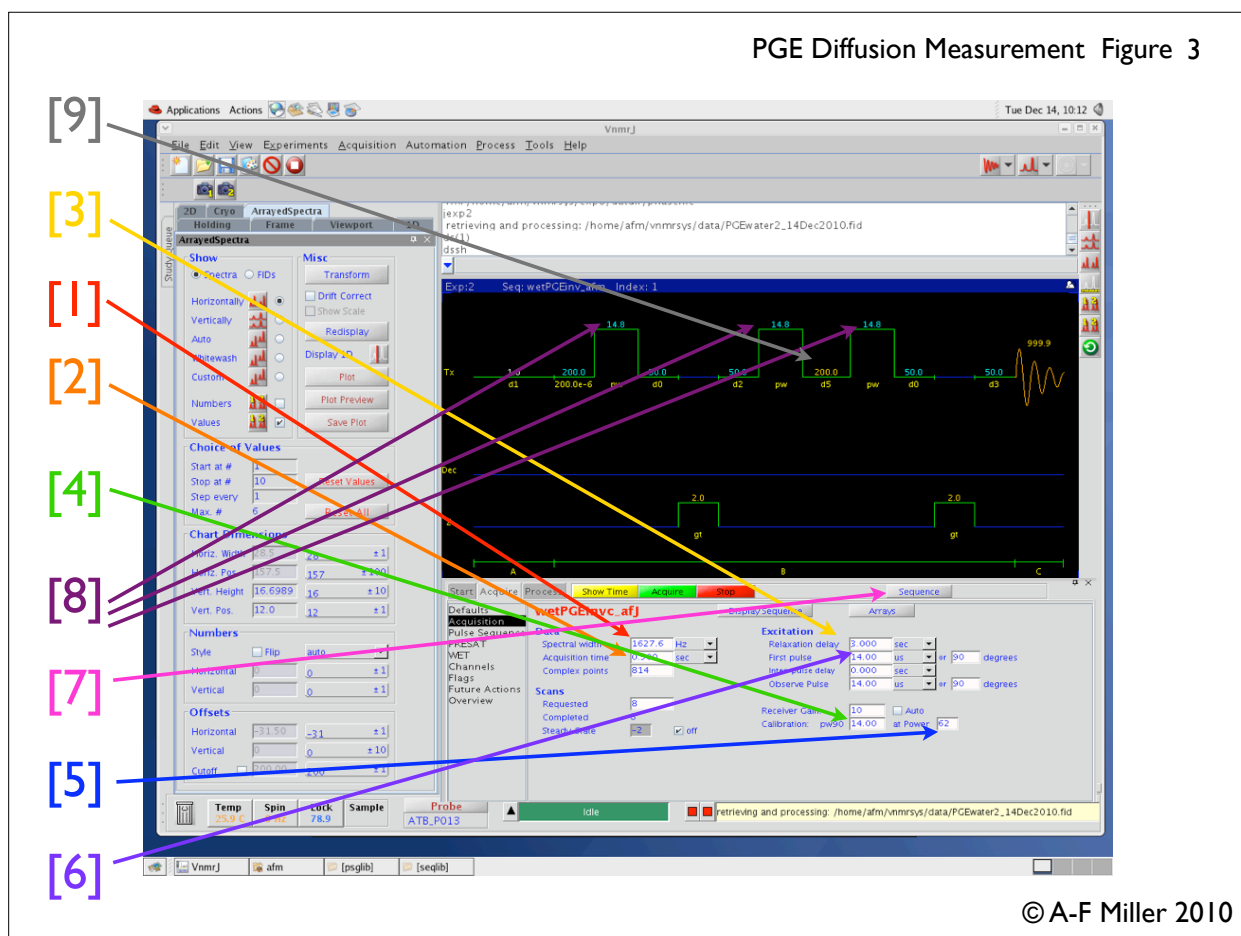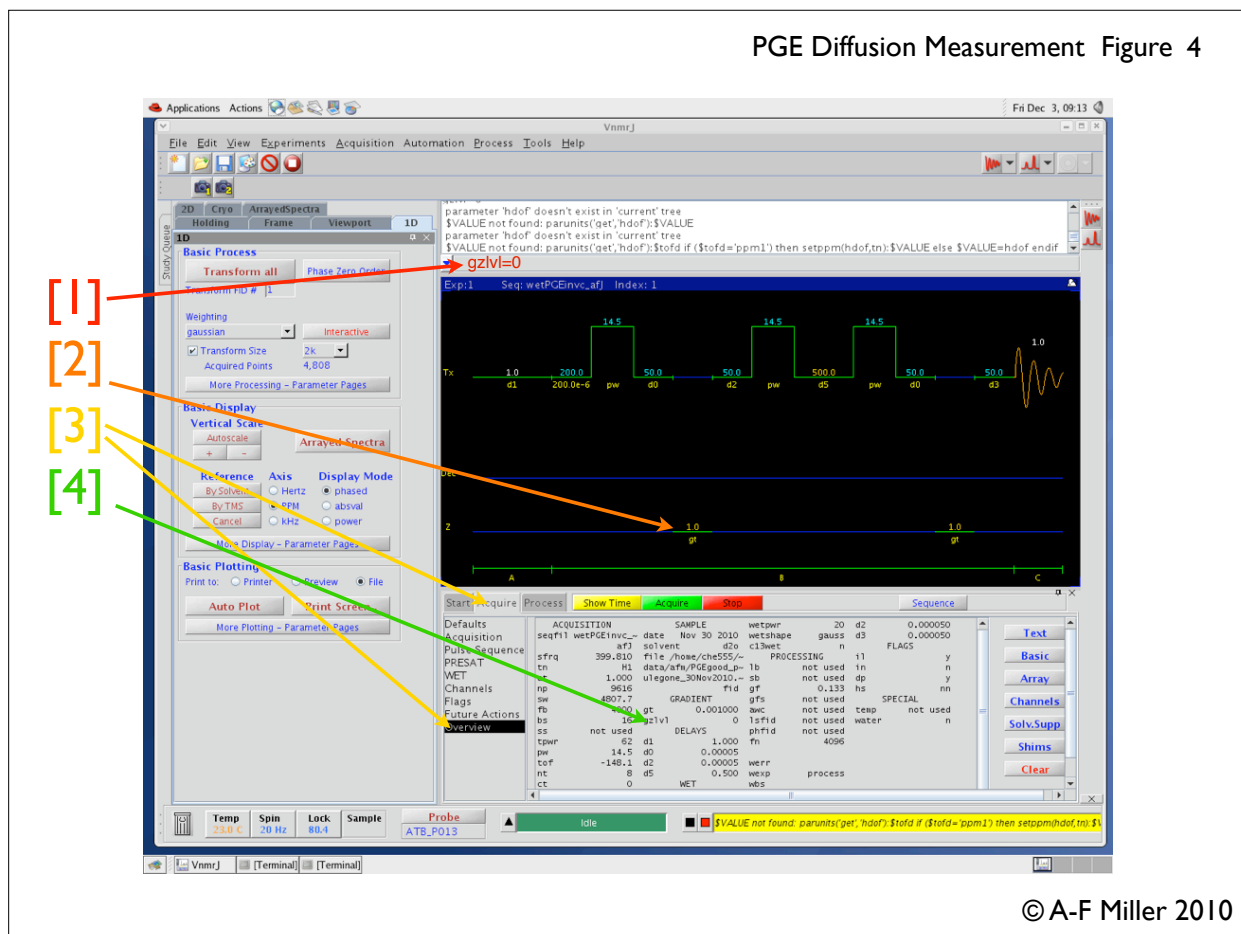# measurement of diffusion coefficient
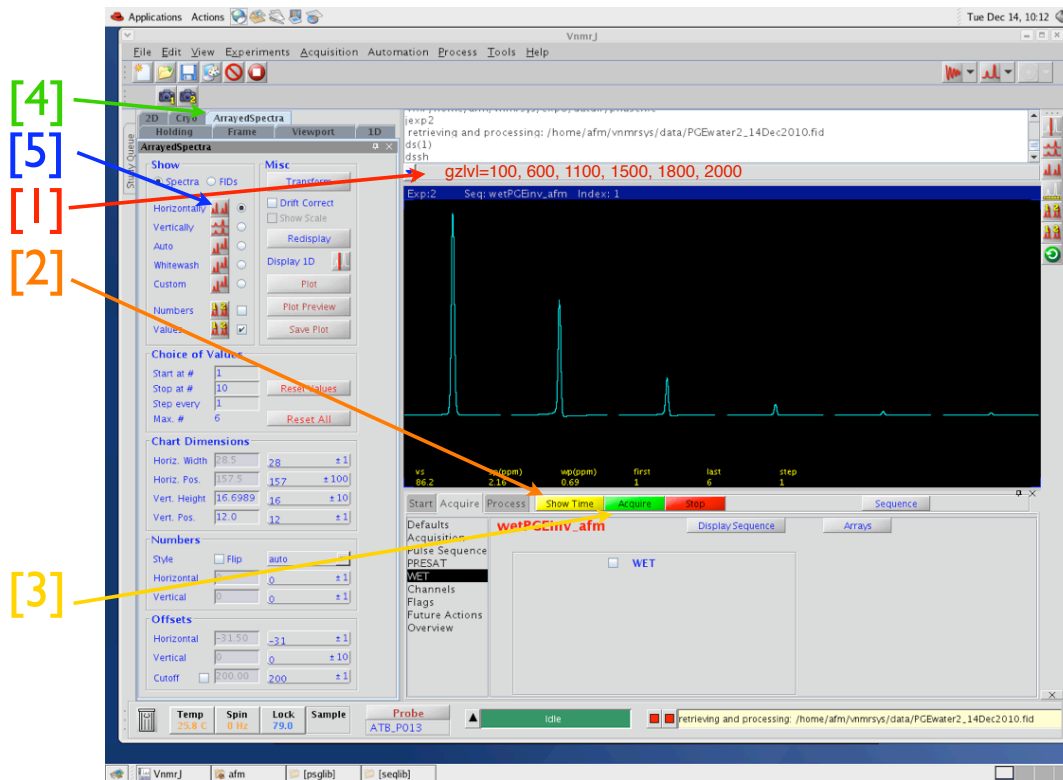
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Water signal amplitude v s. gzlvl²
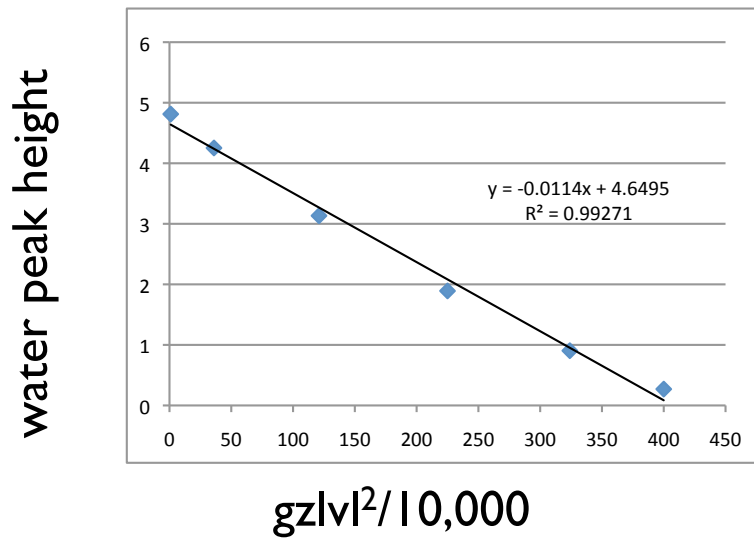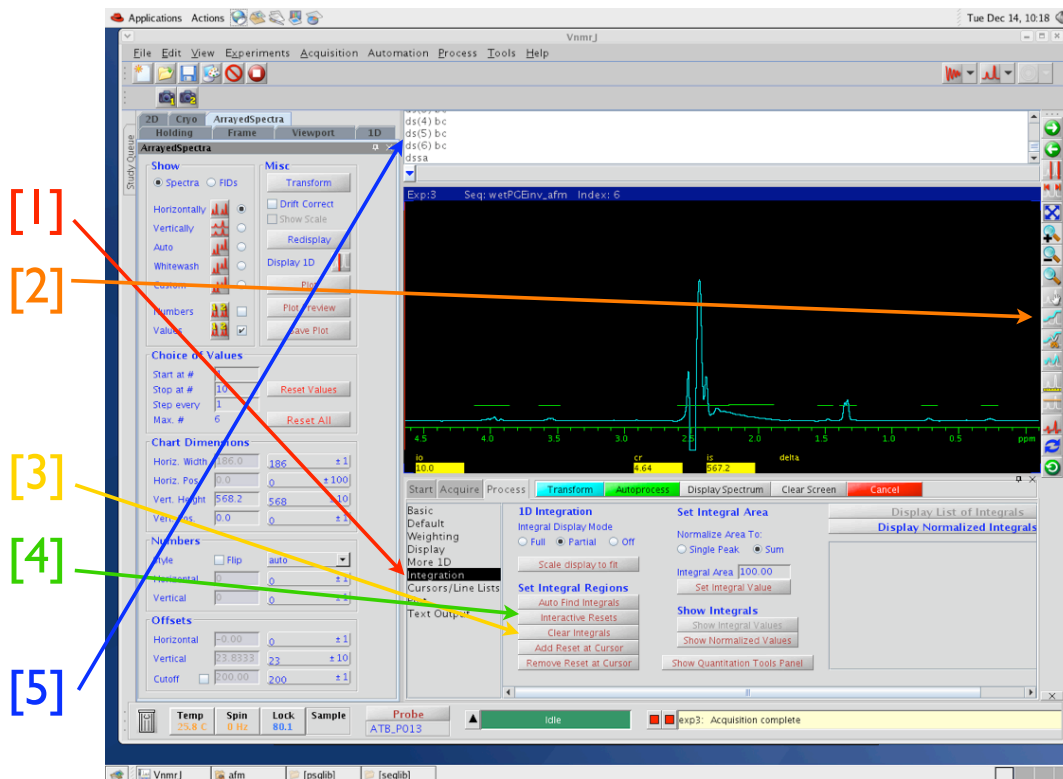
$y = -0.0114x + 4.6495$
$R^2 = 0.99271$

water peak height
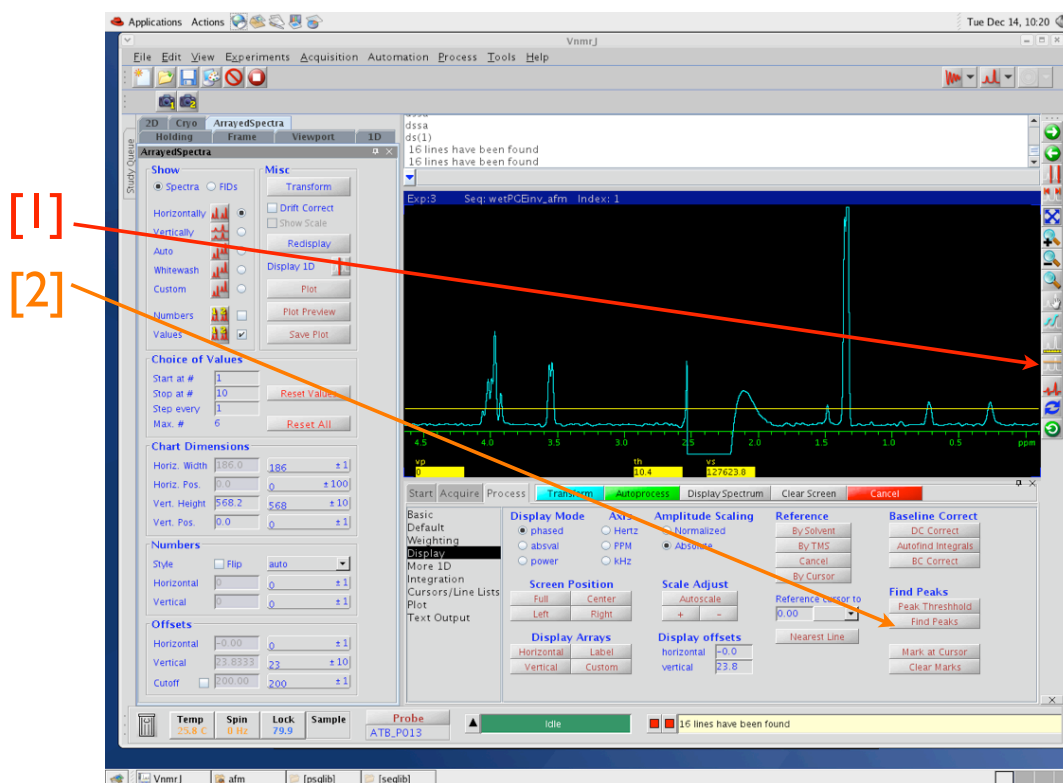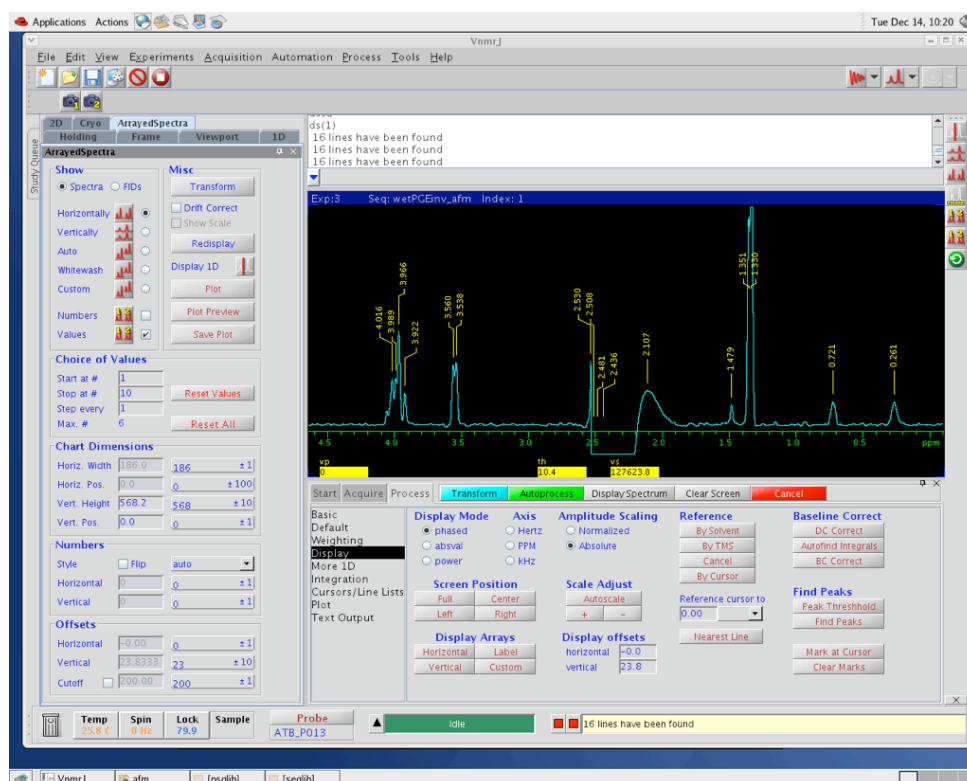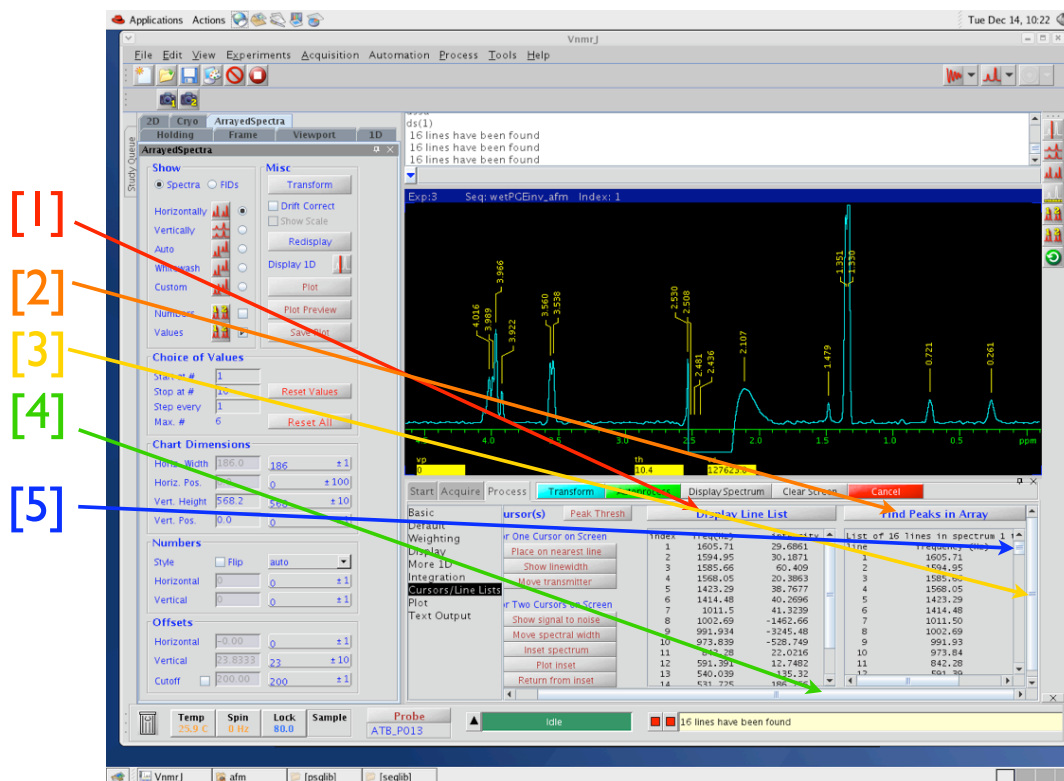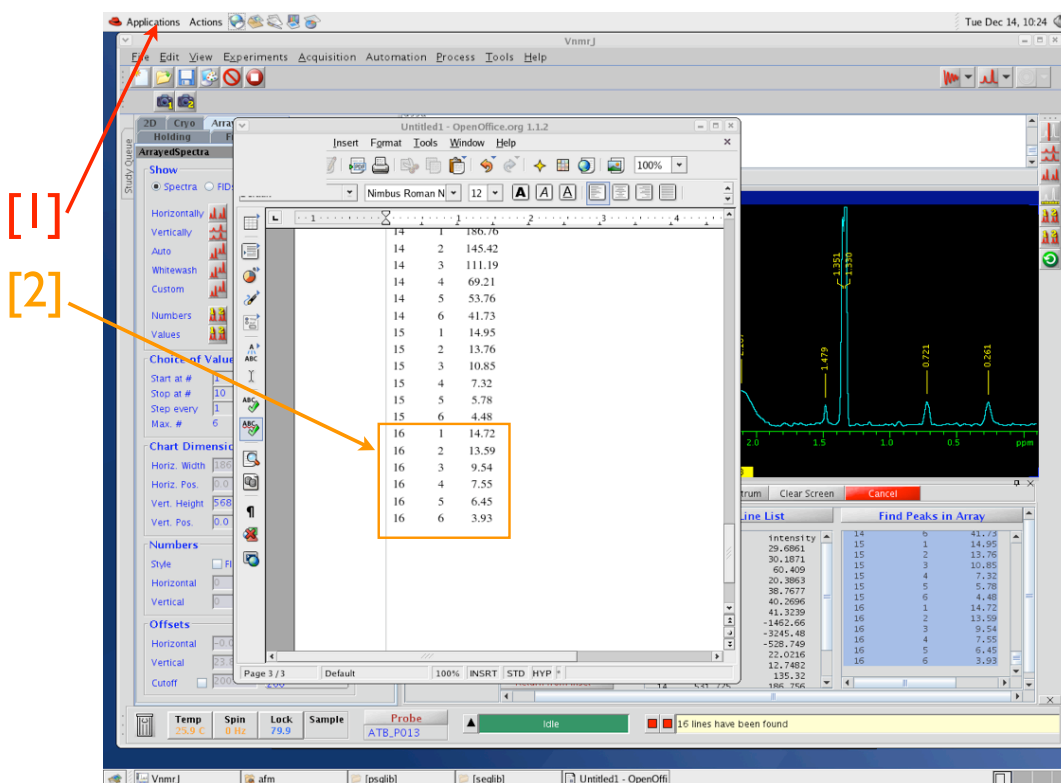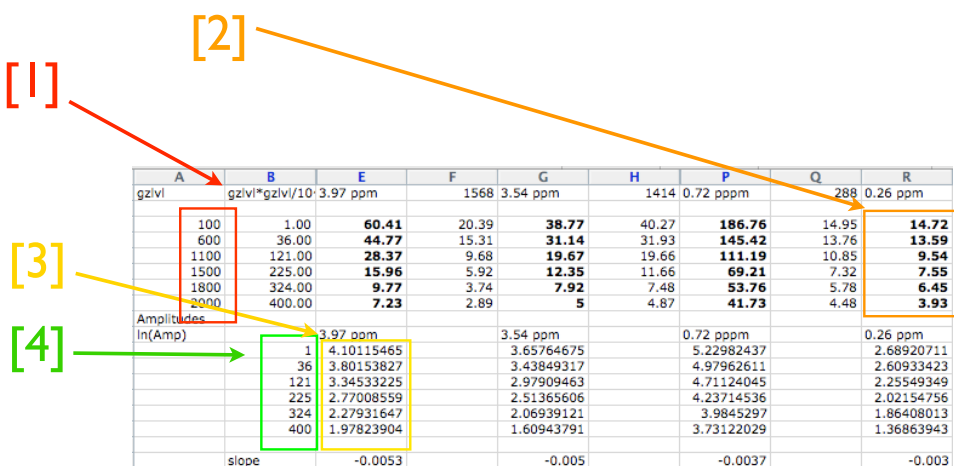
gzlvl²/10,000

Other signal amplitudes v s. gzlvl^2

Chart with data series:
- 3.97 ppm (▲): $y = -0.0053x + 4.0214$, $R^2 = 0.99463$
- 3.54 ppm (✕): $y = -0.0050x + 3.6297$, $R^2 = 0.9977$
- 0.72 ppm (✳): $y = -0.0037x + 5.1555$, $R^2 = 0.98913$
- 0.26 ppm (●): $y = -0.0030x + 2.6961$, $R^2 = 0.9658$
- Linear(3.97 ppm)
- Linear(3.54 ppm)
- Linear(0.72 ppm)
- Linear(0.26 ppm)

y-axis: peak heights
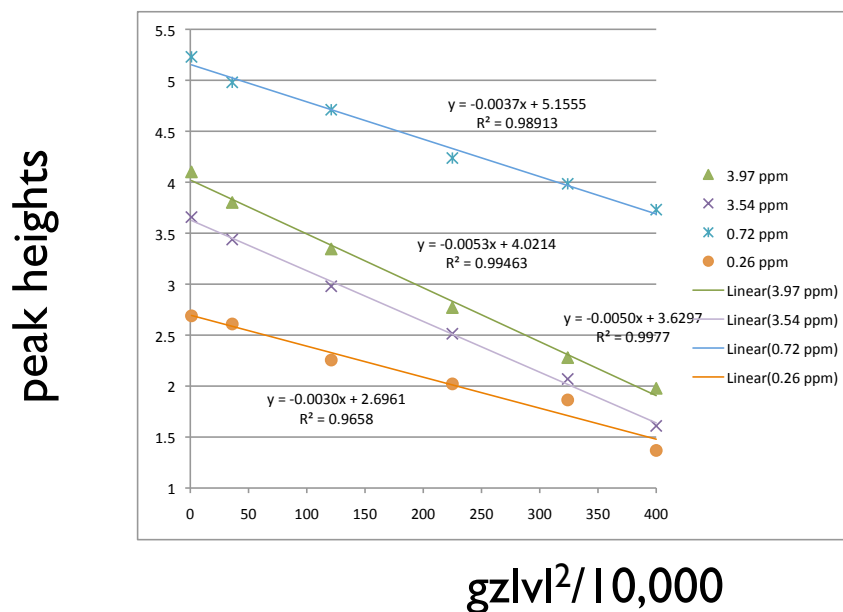
x-axis: gzlvl^2/10,000

Calculation of diffusion constants via calibration on solvent water.

resonance	slope x 10,000	D
3.97 ppm	-0.0053	8.4 x 10^{-10} M^{2}s^{-1}
3.54 ppm	-0.0050	7.9 x 10^{-10} M^{2}s^{-1}
0.72 ppm	-0.0037	5.8 x 10^{-10} M^{2}s^{-1}
0.26 ppm	-0.0030	4.7 x 10^{-10} M^{2}s^{-1}

vs. water, slope x 10,000 = -.0114 and D = 1.8 x 10^{-9} M^{2}s^{-1}