

Laboration 8b

Kinetics

KR

To consider

NMR kinetics

For an NMR kinetics experiment, one obtains series of spectra over a period of time. There are two possible ways to do this: with the **kinetics** program made from multizg or in pseudo 2D mode from a T_1 -experiment, **A_KINETIC** or **A_KINETIC_DEC**

Time Resolution

For either method, one needs to consider the number scans taken for each spectrum since it will affect the time resolution of your experiment. That is, if your delay between spectra is only 5 minutes and each spectrum requires 5 minutes then your time resolution would be poor. Limit the number of scans (ns) to be as small as necessary for adequate signal-to-noise to improve time resolution.

Temperature

Usually in a kinetics experiment it is very important to carefully regulate the temperature. Before you insert your sample, set the desired temperature and use a dummy sample of similar characteristics to pre-shim the magnet and allow enough time for the probe to equilibrate, about 15 minutes. Set all the acquisition parameters while you wait. Insert your sample and wait again for the sample to equilibrate, at least 5 to 10 minutes. The larger the temperature difference between room and probe, the longer the equilibration period should be.

Quantitative integration

The spectrum should be centered in the region of interest and you should leave at least 15% of free baseline around both sides of the spectrum. Record a new spectrum to verify the new parameters.

Digital resolution

For accurate integration the digital resolution should be very good. Each peak should be covered by at least four acquired data points in order to properly represent its area. For this reason it is usually a good idea to increase the number of points, the acquisition time can be up to 5 or 6 seconds for ^1H NMR. The recycle time (the time between each scan, usually $d1+aqc$) is also important. When you are using 30 degree pulses (the default) the recycle time should be at least $2 \cdot T_1$ of the slowest relaxing nuclei. Medium size organic compounds have T_1 values from 0.1 to about 3 seconds. Therefore you may need to increase the relaxation delay $d1$ to increase the recycle time if slowly relaxing signals like methyl groups are important for your experiment. Especially if you are using an internal reference for integration, such as a known concentration of a small molecule like acetone, TMS or dioxane, it is important to use long recycle times as the relaxation times for these molecules can be very long (several seconds).

WAY 1

Kinetics from multizg exp, linear sampling of kinetic data.

The kinetics program results in a series of spectra that are linearly spaced over time, i.e., each spectrum is separated from the next by the same amount of time. The start time is set to the official time, so that the timing should be 100% accurate. Kinetics is simple: it simply increments the experiment numbers a specified number of times, recording spectra according to the parameters in the first of the stacked experiments.

Practical

1. Use a dummy sample of similar characteristics to pre-tune, pre-lock and pre-shim the magnet. Depending on the kinetics of reaction can you get a measure-point at zero can by taken up a spectrum on the sample before adding the reactant.
2. Set up and run a experiment on the desired nucleus according to "Bruker run manual for 500 MHz NMR", **edc**, **getprosol**, **atma**, **lock**, **topshim**, **rga**, **zgefz**, **apk**. If necessary optimize **sw** and **o1p**.

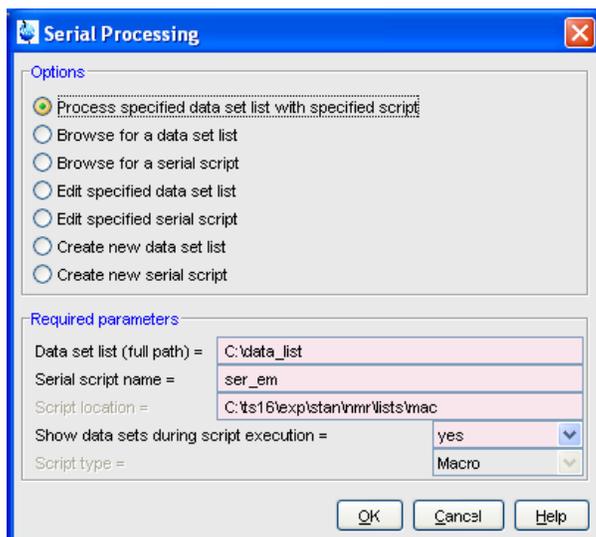
Experiment Setup

1. **wrpa 2**
2. **re 2**
3. insert the sample or eject it and add the reactant and then insert (time zero)
4. When running kinetics at higher or lower temperature than room temperature you have to take in consideration the time it takes for the sample to equilibrate. One solution is to pre-warm/pre-cold the sample to the same temp as you decided to run the kinetics on.
5. **lock** (click on lock=on on the BSMS keyboard)
6. **topshim** (if the reaction time allows)
7. **rga**
8. **kinetics** (a modification of multizg)
Answer the questions, how many experiments and how long the delay time (in seconds) you want to have between the experiments.
Kinetics starts from the experiment you are in.
9. To maintain good homogeneity (shim) one can shim manually after each experiment or use autoshim. To use autoshim, simply press the autoshim button at the bottom of the BSMS (lock) keypad

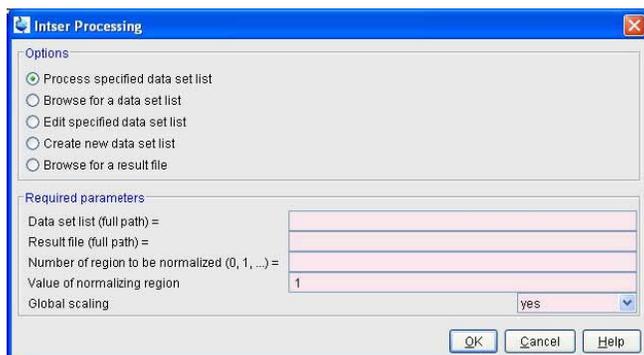
PROCESSING

(Topspin 2.0)

1. Process the first spectra
efp, apk, abs n
2. Integrate the desired peaks in the first spectra, save.
3. The remaining spectra in the kinetic run can be processed and integrated in serial.
4. **serial** or (Processing → Serial Processing ..)



5. Select “create new data set list”, OK, next window click OK
6. In the “Find data” window enter Name and User and enable the checkbox. OK
7. You will get a “search result file”. Mark the desired spectra, click save selection, and give it a File name (i.e *XXX-list.txt*). The name should have the end **.txt**. Navigate to the desired store directory. OK
8. Browse for a serial script (script type=Macro)
If you mark and click *edit* can you see what the macro contains. If you do not find anyone you want to use, create or edit one and save in another name.
9. “Process specified data set list with specified script”, OK

10. **intser** or (Analysis→ Integration→”Integrate a list of spectra”)

11. Before you “Process specified data set list” You have to fill in the “Required parameters”

- If the full path for the Data set list isn't in the window write it in or browse for the list you created in the serial window.
- Give a full path for the Result file. You can copy-paste the data set list path and then change the filename to *XXX-result.txt* instead of *XXX-list.txt*.
- Number of region to be normalized, observe that the first integrated peak from left hand side is numbered as 0 (zero).
- Number of normalizing region, i.e 1
- Global scaling: Takes the value *yes* or *no*.

For **yes**, all integrals of all spectra in the list will be scaled relative to the normalization region of the reference spectrum.

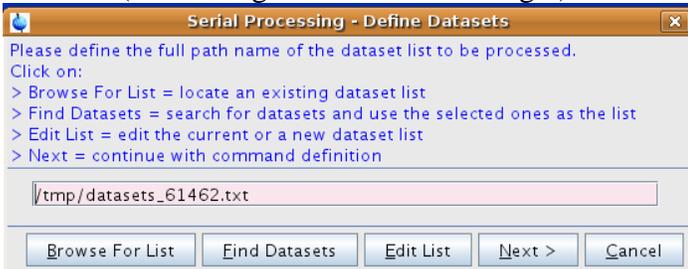
For **no**, all integrals of one spectrum will be scaled relative to the normalization region of the same spectrum (i.e. internal standard). The normalization region number and value are same for each spectrum (the specified values)

12. Process specified data set list, OK

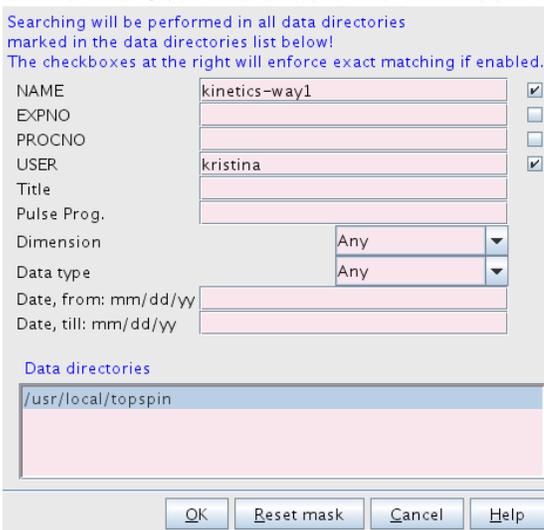
- The integration result is displayed in a text window. In addition it is stored in a text file whose full pathname was specified in the *intser* dialog. Lines beginning with a # are comment lines. The format is suitable to be imported into a spreadsheet program such as Excel for further processing.

PROCESSING (Topspin 2.1)

1. Process the first spectra
efp, apk, abs n
2. Integrate the desired peaks in the first spectra, save.
3. The remaining spectra in the kinetic run can be processed and integrated in serial.
4. **serial** or (Processing → Serial Processing ..)



5. Browse for a list if you have one.
6. If you want to create a new list click “Find data”. In the “Find data” window enter Name and User and enable the checkbox. OK



7. You will get a “search result file”. Mark the desired spectra, click save selection. The data list will be stored as datasets_#number.txt in /tmp. OK

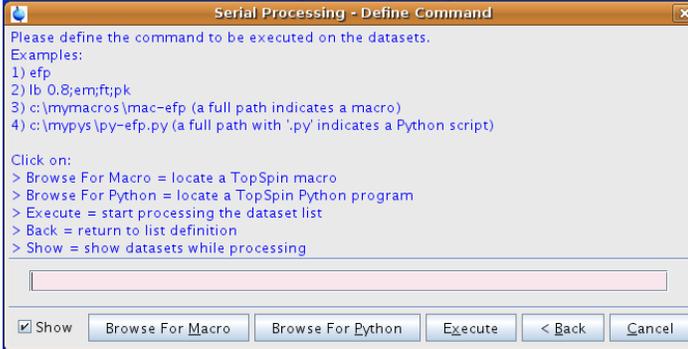
Found: 10 Data Sets.
Please right-click in a list for more options!

kinetics-way1	1	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:06:37
kinetics-way1	2	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:11:37
kinetics-way1	3	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:16:37
kinetics-way1	4	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:21:37
kinetics-way1	5	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:26:36
kinetics-way1	10	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:42:49
kinetics-way1	11	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:44:49
kinetics-way1	12	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:46:49
kinetics-way1	13	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:48:49
kinetics-way1	14	1	/usr/local/topspin	kristina	1	zg30	2009-01-21 11:50:49

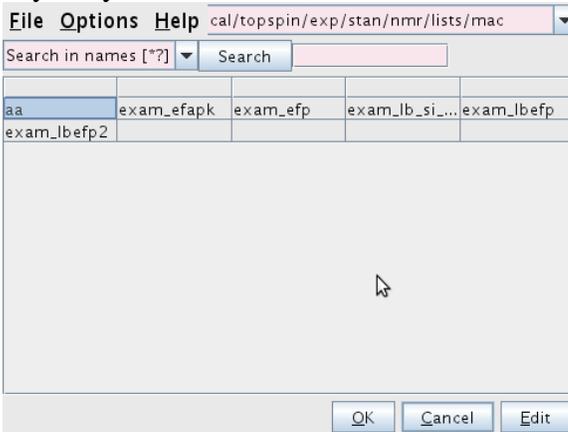
8. If you want to look on the list Edit it, otherwise clic Next



9. Browse for a serial script (script type=Macro)

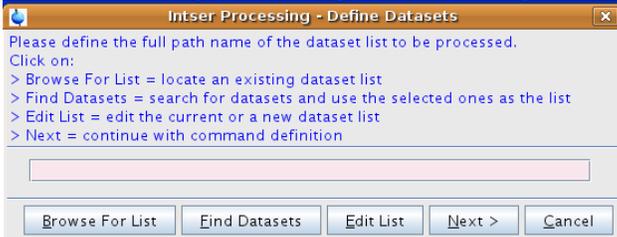


10. If you mark and click *edit* can you see what the macro contains. If you do not find anyone you want to use, create or edit one and save it in another name



11. “Process specified data set list with specified script”, OK

12. **intser** or (Analysis→ Integration→”Integrate a list of spectra”)



13. Before you “Process specified data set list” You have to fill in the “Required parameters”

- If the full path for the Data set list isn't in the window write it in or browse for the list you created in the serial window, i.e /tmp/datasets_61462.txt.
- Number of region to be normalized, observe that the first integrated peak from left hand side is numbered as 0 (zero).
- Number of normalizing region, i.e 1
- Global scaling: Takes the value *yes* or *no*.

For *yes*, all integrals of all spectra in the list will be scaled relative to the normalization region of the reference spectrum.

For *no*, all integrals of one spectrum will be scaled relative to the normalization region of the same spectrum (i.e. internal standard). The normalization region number and value are same for each spectrum (the specified values)

14. Process specified data set list, OK

15. The integration result is displayed in a text window. In addition it is stored in a text file in /tmp/IntserResult_#number.txt. Lines beginning with a # are comment lines. The format is suitable to be imported into a spreadsheet program such as Excel for further processing.

```

File Edit Search
1 # Result of 'intser'
2 # Date/time = Mon Feb 02 14:09:25 CET 2009
3 # Data set list (full path) = /tmp/datasets_44930.txt
4 # Normalization region = 0
5 # Normalization value = 1.0
6 # Global scaling = yes
7
8 # --- Integral info ---
9 # A 1.0 #regions in PPM
10 # # low field high field bias slope
11 # 8.486108004348232 8.42167286384445 -0.0 -0.0 # for region 1
12 # 6.121338347859393 6.008576851977725 -0.0 -0.0 # for region 2
13
14 # Spectrum#; Integral 0; Integral 1;
15 0;1.0;0.9685536700838102;
16 1;0.9896195130939764;0.9635399508948641;
17 2;1.0007581414501163;0.9678893784384144;
18 3;0.9935265643771737;0.9654802605713474;
19 4;0.9984837847362098;0.9677391726884871;
20
1 : 1

```

WAY 2

Kinetics as a pseudo 2D (T_1 -exp), non-linear sampling of kinetic data.

The 2D mode allows non-linear spacing between spectra and the timing between spectra is exact. It uses the T_1 subroutines for processing so getting intensity data for the whole series is simple. Like a T_1 experiment, a list of delays must be specified, which in this case is the time between spectra. For a constant time between spectra, the list needs to have only one value. The program “loops” if the number of spectra exceeds the number of values in the list.

Experiment setup

1. **edc** or **new**
choose the parameter set *A_KINETIC* or *A_KINETIC_DEC*
(These are modified from a T_1/T_2 2D exp.)
2. **getprosol**
3. **edlist** If you want to make a list of your own with desired delays.
4. In AcqPar
 - a. Check the parameters **ns**, **sw**, **o1p**, **d1**.
 - b. **TD F1** to the same value as you have entrees in the VD-list
 - c. **FnMODE** to **QF**
 - d. **VD list** to the name of your choose, the one you created or an old one for example kinetic_delay (you can modify it)
5. In Propar
 - a. **SI in F1** make equal to or slightly greater to TD in F1/ VD-list enteries (restriction must be a multiple of 4, e.g 1, 2, 4, 8, 16)
 - b. Set PH_mod to pk in the F2 dimension.
 - c. Click the Spectrum tab to save
6. To calculate the resulting experiment time for current data use the command **expt** or click on the button  in the upper toolbar.
7. **rga**
8. **zg**
9. To maintain good homogeneity (shim) one can shim manually after each experiment or use autoshim. To use autoshim, simply press the autoshim button at the bottom of the BSMS (lock) keypad.

PROCESSING

Processing is similar to T1 experiments. You must first phase all the spectra.

10. Type **rsr #** to read in one 1D spectrum (if there are 15 delay values, then # may range from 1 to 15).

11. **ef**

12. **apk**

13. Go to manually phasing 
save the phasing to both 2D and 1D by clicking on , 

14. Select which peaks are to be analyzed, based on intensity or area.

Peak pick (intensity) 

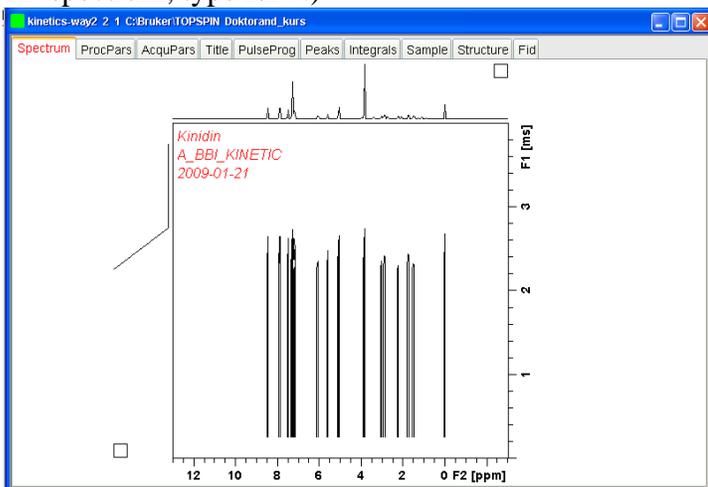
Add one or more peaks manually by clicking  and select peak with cursor and left click. Click  to export to the relaxation analysis routine.

Integrate (area) 

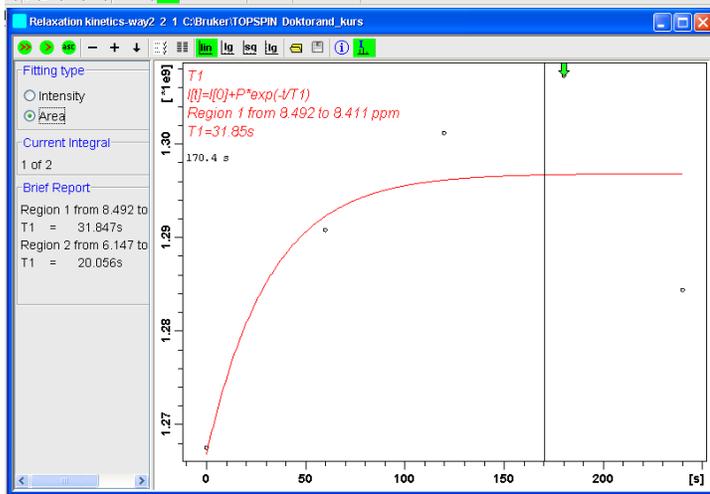
Click  to define beginning and end of integral; press left button and drag to define. After adjusting integral, export to relaxation module by clicking  and then select “Export Regions to Relaxation Module”.

15. Click  to close the 1D spectrum and return to 2D.

16. Type **xf2** to transform all the spectra. (If at any time you wish to return to the above 1D spectrum, type **rsr #**.)



17. Type **t1/t2** to enter the relaxation sub-routine.



18. Your points should be automatically picked. To pick them manually, click . To adjust the settings, click . If your points are not all being picked, you may also need to increase the number of drift points, and then re-pick.

19. Click to calculate the first peak, to calculate all peaks for T1.

20. To delete a particular, possibly outlying point, right-click over the point and select delete. If you have picked more than one peak in your spectrum, click + or – to change to the next peak.

21. Click to generate a report.

This report is saved as a text file in your process number directory of the NMR data or save it in a name and place of your own. It can easily be cut-and-pasted into Excel for further processing.

tau	ppm	integral	intensity
100.000u	8.447	1.2676e+009	7.6457e+007
60.000s	8.447	1.2909e+009	7.3445e+007
120.000s	8.447	1.3013e+009	7.3862e+007
180.000s	8.447	1.3074e+009	7.6773e+007
240.000s	8.447	1.2844e+009	7.6061e+007

tau	ppm	integral	intensity
100.000u	6.062	1.3051e+009	1.7323e+007
60.000s	6.063	1.3044e+009	1.7273e+007
120.000s	6.062	1.3027e+009	1.7424e+007
180.000s	6.062	1.3071e+009	1.7231e+007
240.000s	6.063	1.3028e+009	1.7364e+007