Kinetic Profiling by NMR

This document summarises a procedure for obtaining kinetic (time course) data with NMR on Bruker instruments using TopSpin. This provides a means of monitoring reactions as a function of time in the NMR tube and potentially for determining kinetic parameters from the time course data. Ideally, kinetic data is acquired with deuterated solvent, or a small quantity of deuterated solvent. This ensures the spectrometer remains locked and stable throughout, and solvent signals do not interfere with peak integration or spectral quality.

There are three procedures for **acquiring kinetic data** using TopSpin. Ensure you are following the correct procedure for your requirements.

- **Method 1**: Manual mode, one nuclei only (i.e., ^{1}H NMR)
- **Method 2**: Manual mode, interleaved nuclei (i.e., ^{1}H and ^{19}F NMR)
- **Method 3**: Using IconNMR, one nuclei only (i.e., ^{1}H NMR)

There are two procedures detailed for **processing kinetic data** using either TopSpin or MestreNova. For Method 2, MestreNova is recommended. Method 1 and Method 3 can be processed using TopSpin or MestreNova.

Acquiring kinetic data using TopSpin

Method 1: Obtaining kinetic data outside of IconNMR (manual mode, one experiment only)

Follow this procedure if you wish to run one experiment only periodically outside of IconNMR.

1. First lock, tune, shim and run initial spectra of your starting materials before the reaction to make sure you know exactly where they appear in the spectrum with the same solvent that you use in the reaction under the same conditions, e.g. temperature, same spectrometer.
2. Having optimised the spectra of your starting materials alone (tuning the probe and shimming), quickly run the first spectrum of your reaction after mixing noting the time you started (the command **dpa** will display the exact end time of each acquisition and these times can be used in generating time course profiles). If your reaction is fairly slow you may have time to do some quick shimming beforehand (command **topshim 1dfast**); re-tuning is not necessarily required.
3. Having run the first reaction spectrum, increment experiment number (**ix**, or **iexpno**), and use the command **multi_zgvd** (answer the following questions). If you require no delay between experiments, use the command **multizg**:

*Enter fixed delay (in seconds, for **multi_zgvd** only):*
e.g. **600** (10 minutes for the spectrometer to wait until next acquisition)

Number of Experiments (for both **multizg_vd** and **multizg**)
e.g **30**

The program will set up all the experiments then tell you how long your kinetic run should take. Note if you would like a spectrum running every five minutes and your experiment takes 60 seconds, the fixed delay time should be set to 240 seconds.

Method 2: Obtaining kinetic data outside of IconNMR (manual mode, interleaved nuclei)

Follow this procedure if you wish to run interleaved nuclei experiments periodically outside of IconNMR. This procedure does not allow the spectrometer to tune and match the nuclei between time points therefore certain combination of experiments are not feasible on specific probes. For example, running ^{19}F and ^{31}P experiments is not possible on a BBFO probe. Ask the NMR staff if you are unsure.

1. First lock, tune, shim and **run all initial spectra** of your starting materials **before** the reaction to make sure you know exactly where they appear in the spectrum with the same solvent that you use in the reaction under the same conditions, e.g. temperature, same spectrometer (experiments 1 to 3 below).
2. Having optimised the spectra of your starting materials alone (tuning the probe and shimming), quickly run all the first spectra of your reaction after mixing (experiments 4 to 6 below) noting the time you started (the command **dpa** will display the exact end time of each acquisition and these times can be used in generating time course profiles). If your reaction is fairly slow, you may have time to do some quick shimming beforehand (command **topshim 1dfast**); re-tuning is not necessarily required. If you are running more than two interleaved nuclei, ensure the experiment numbers are consecutive as shown below.

3. Having run the first reaction spectrum, open the first experiment run of the kinetic study (in this case, experiment 4) and use the command **multizgs** (answer the following questions).

Enter 1st experiment to copy:
e.g. **4** (experiment 4 is the first nuclei at time point 0 here)

Enter last experiment to copy:
e.g **6**

In which experiment should the first experiment by run:
e.g **7** (ensure there are no numbers in the folder that are larger than this number)

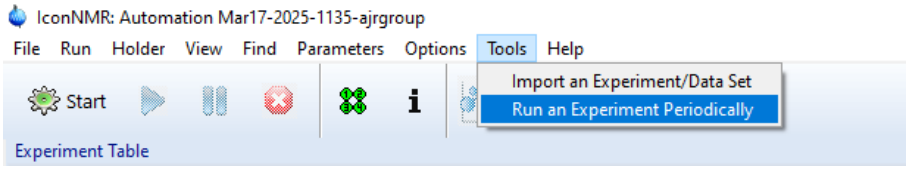
Enter number of times to repeat the experiments:
e.g **20** (how many time points to be acquired. Note there is no delay between experiments)

Do you want to (r)un the experiments, or just (c)reate them?:
e.g **r** or **c** (if you wish to start the run immediately, type **r**. If c is selected, every experiment must be queued manually)

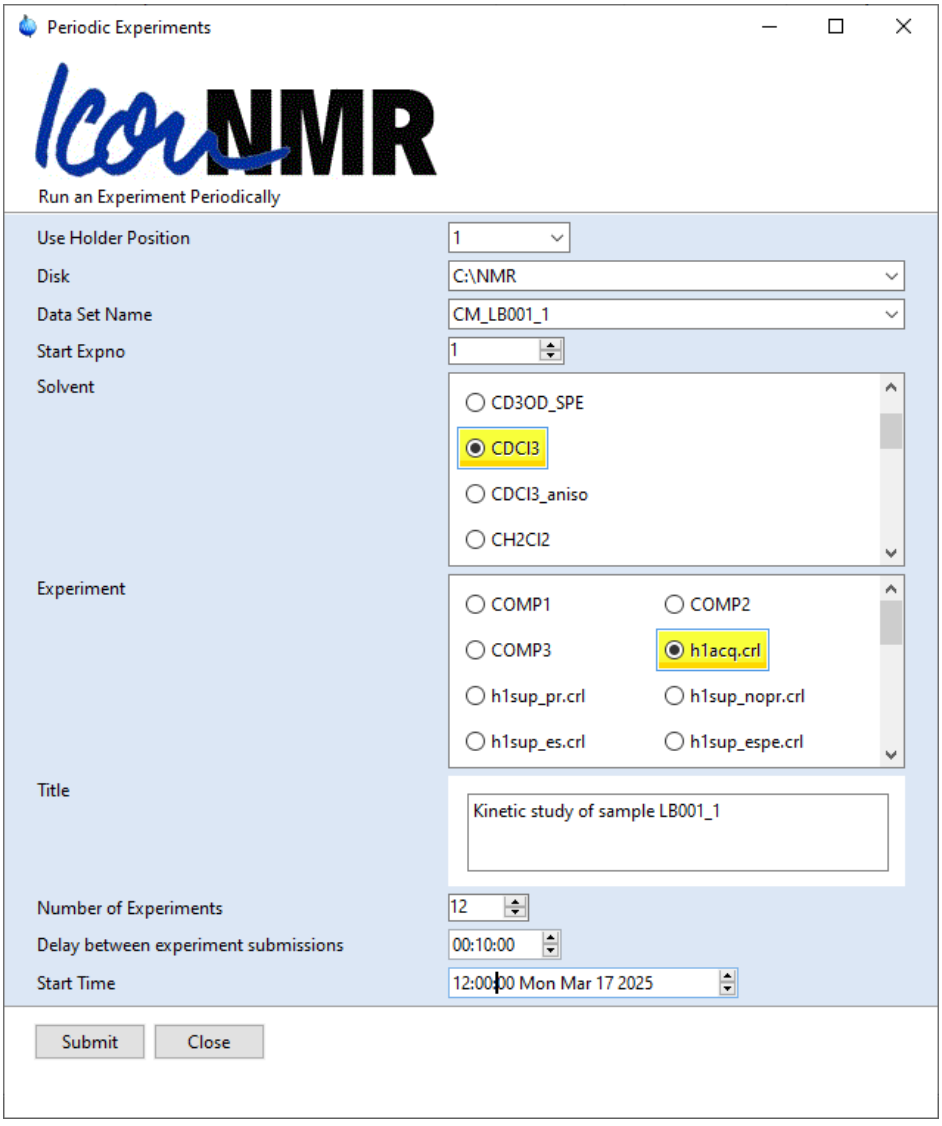
The program will set up each experiment as it runs. It will not state how long the kinetic run should take, nor queue anything in the spooler. It is highly recommended to use the available 'kinetic time course in progress' sign available in the lab.

Method 3: Obtaining kinetic data using IconNMR (one experiment only)

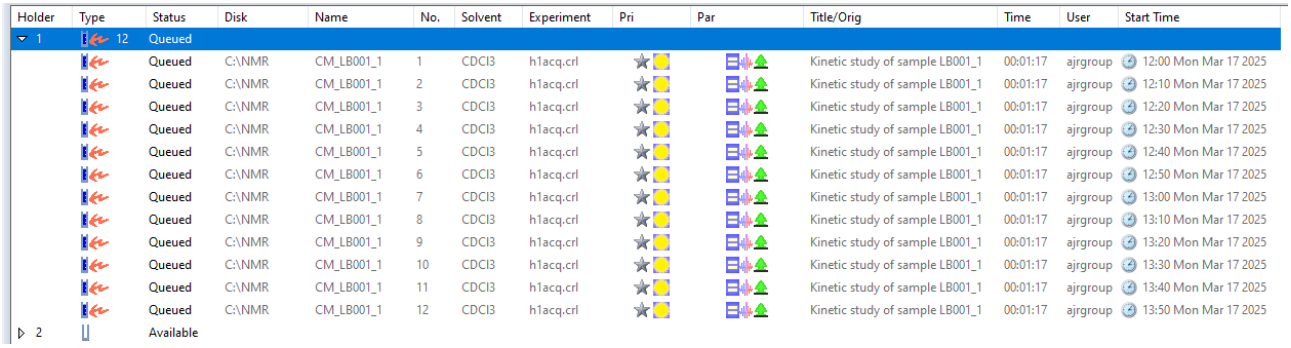
1. Open Icon NMR, change user to your research group and ensure IconNMR is running.
2. In the Menu bar, Click Tools – Run an Experiment Periodically:



3. A top-up window titled 'Periodic Experiments' will appear. Fill out all sections with the relevant information. Use the drop-down option to select the correct *Disk*. Set *Start Expno* to 1. Set the *number of experiments* and *delay between experiment submissions* (time for the spectrometer to wait until next acquisition). If you wish for the experiment to start ASAP, leave the *Start time* as is. If not, set the time in a 24-hour format to when you wish the experiments to begin. Once all sections are complete, click *Submit*.



Once submitted, IconNMR will automatically populate the specified position holder when the relevant experiment and experiment times. To determine what time your kinetic run will finish, add the experiment time to the start time of the last experiment.



Holder	Type	Status	Disk	Name	No.	Solvent	Experiment	Pri	Par	Title/Orig	Time	User	Start Time
1	12	Queued											
		Queued	C:\NMR	CM_LB001_1	1	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:00 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	2	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:10 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	3	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:20 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	4	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:30 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	5	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:40 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	6	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	12:50 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	7	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:00 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	8	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:10 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	9	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:20 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	10	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:30 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	11	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:40 Mon Mar 17 2025
		Queued	C:\NMR	CM_LB001_1	12	CDCl3	h1acq.crl			Kinetic study of sample LB001_1	00:01:17	ajrgroup	13:50 Mon Mar 17 2025
2		Available											

Processing kinetic data using TopSpin

1. Process your first spectrum using **efp** and phase it perfectly, manually. You need all subsequent spectra in the kinetic run to be phased in exactly the same way, then the baseline corrected. Take note of the PHC0 and PHC1 values under the PROCPARS tab.
2. Use the **multicmd** command to do this, which asks you the number of experiments required, and the commands for processing.

Choose procedure for 'multicmd':
NORMAL

Enter number of experiments:
e.g. **50** (i.e. 50 time points including experiment 1)

First command:
1 PHC0 [value] (i.e., 1 PHC0 109.06)

Second command:
1 PHC1 [value] (i.e., 1 PHC1 -1.02)

Third command:
efp

Fourth command:
absn

Fifth command:
Leave blank and press enter to finish the command. A confirmation pop-up window may appear depending on the version of TopSpin that is being used. Press OK to confirm.

The macro will phase the specified spectra exactly the same as the first one you phased perfectly, then perform baseline correction (**absn**).

3. Having now obtained all your experiments, please look closely for your signals of interest, those that grow at the expense of those that decrease. You will then need to integrate them in exactly the same way e.g. integral limits and peak positions, for all n experiments.
4. Integrate a good example spectrum of the kinetic run using the command '**.int'**, where you have both starting material and product, '**save regions to intrng'**, then save and return.
5. Write a new integral file with command '**wmisc**' (write new) with filename xxx, enter a new name, i.e, '**CM_int1**'. To be on the safe side, check the integrals are what you want with **rmisc** (read xxx) on another spectrum within your list.
6. When satisfied with your integration of your starting material and product peaks, then go to the first spectrum of the reaction mixture and use the command multi_integ3 (answer list of questions):

   Use EXPNOs (0) or PROCNOs (1):
   0

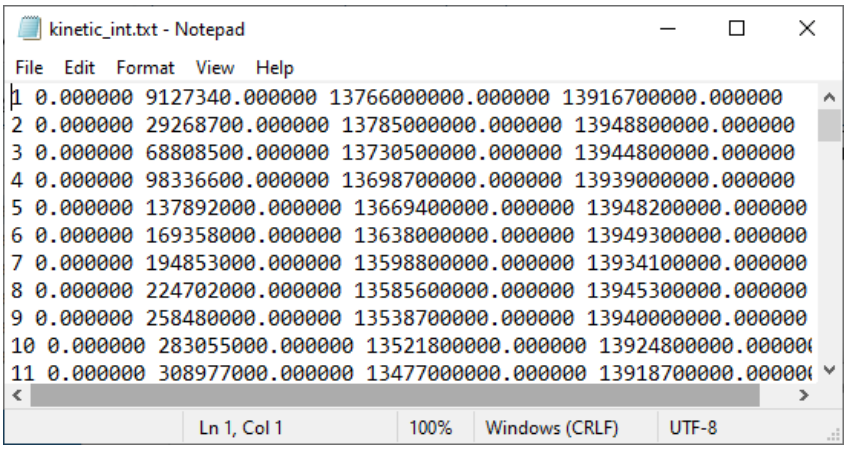
   Enter first experiment No:
   e.g. **1** (if kinetic data is in experiment numbers 1 - 9)

   Enter number of experiments:
   9

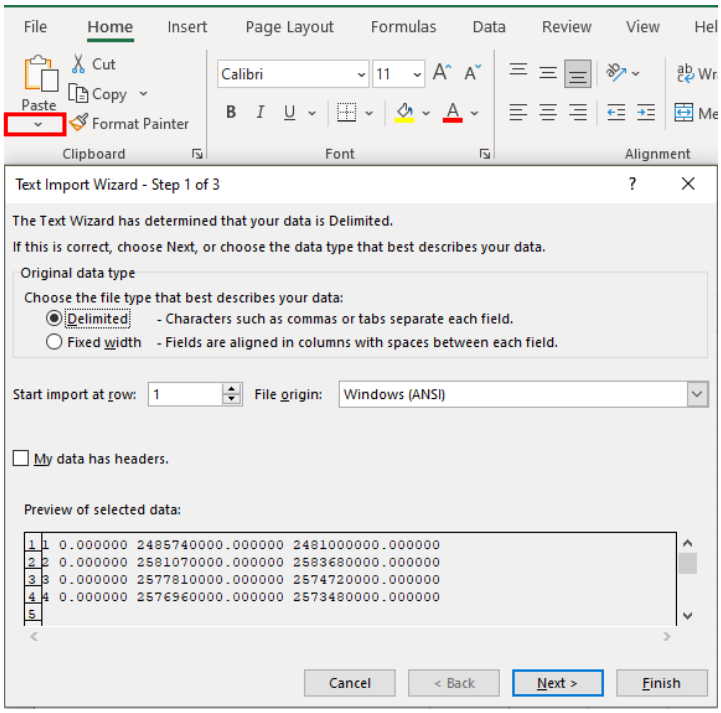
   Enter name of intrng file:
   CM_int1 (i.e., the name of the list saved in step 4)

The program calculates all the integrals in files 1…….n and the results are conveniently presented as a txt file in the experiment you performed the multi_integ3 command in, with the processed spectrum e.g. D:/NMR/data/nmrgroup/file/pdata/1/[file name]_int.txt.

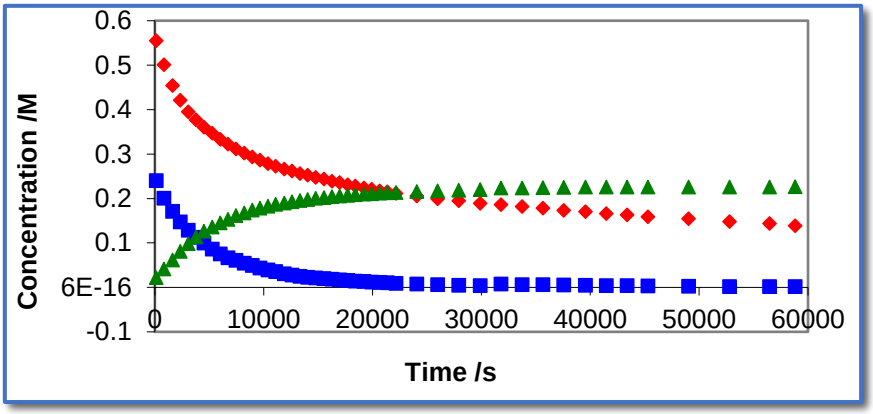
An example of how this text file should appear is shown below;



7. This table of integral values can easily be imported into **Excel** as a Space and Tab delimited file for plotting and for calculations of rate constants, as shown below. In Excel, under *Home* – *Paste* (red box below) – *Use Text Import Wizard*. Ensure 'Delimited' is selected and click Next.
8. On step 2 of 3, ensure the correct delimiter is selected, i.e., 'Space'. Click 'Finish'. This will copy all the kinetic data into separate cells for ease of processing.



9. The data can then be plotted to show integration/concentration vs time (s)



The in-house au program **readdate** may also be used to generate a text file containing the date and time points for a series of spectra of increasing experiment number (expno) and avoids the need to manually extract the time from each spectrum with the dpa command. The generated file (*kineticdates.txt*) will be stored in the expno of the first experiment. Note: this is not part of the standard Bruker installation and will have to be added to your TOPSPIN installation: copy the au program into the directory:

Enter starting expno:
e.g. **2** (if kinetic data is in experiment numbers 1 - 9)

Enter number of experiments to extract dates from:
e.g. **9**

Enter increment between expnos:
1

C:\Bruker\TOPSPIN-VERSION\exp\stan\nmr\au\src\

(or your corresponding Bruker installation path) and, in your first experiment of the kinetic run, use the command **xau readdate** to compile and run the program.

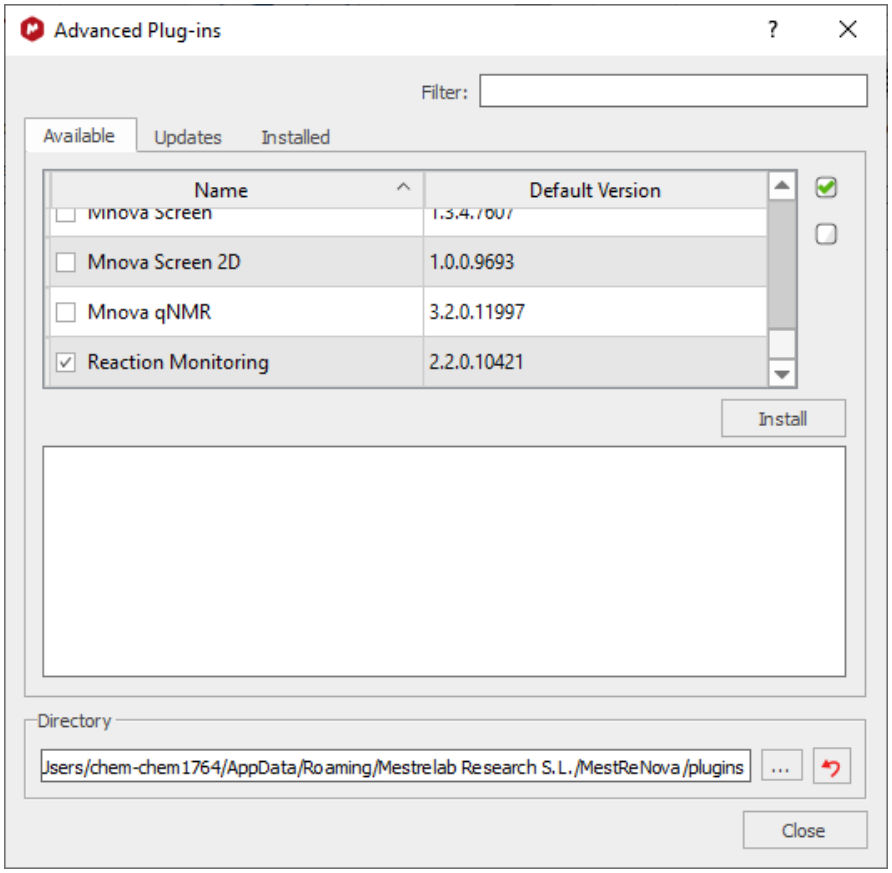
Coral Mycroft, 2026

Processing kinetic data using MestreNova

To process reaction monitoring data with MestreNova, the 'Reaction Monitoring' plug in must be installed. The following procedure is described using the modern MestreNova interface. Adjustments may be required if using the classic interface, or if using a different version of MestreNova.

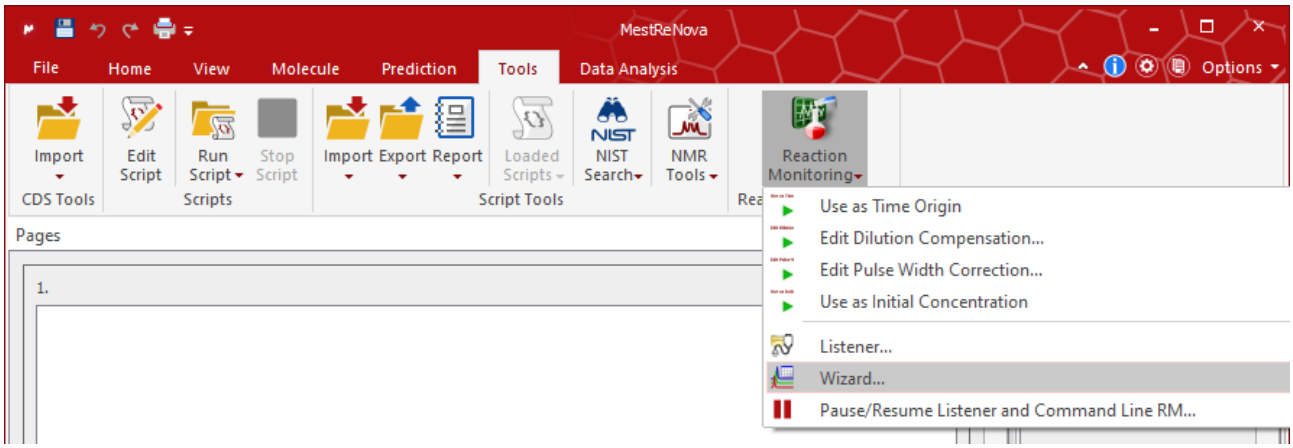
Installing the Reaction Monitoring Plugin

Under 'File', click on Advanced Plug ins in the left-hand panel. Under the 'Available' tab, tick the 'Reaction Monitoring' plug-in and select Install. Close and re-open MestreNova when prompted.



Loading and processing kinetic data using the Reaction Monitoring Wizard

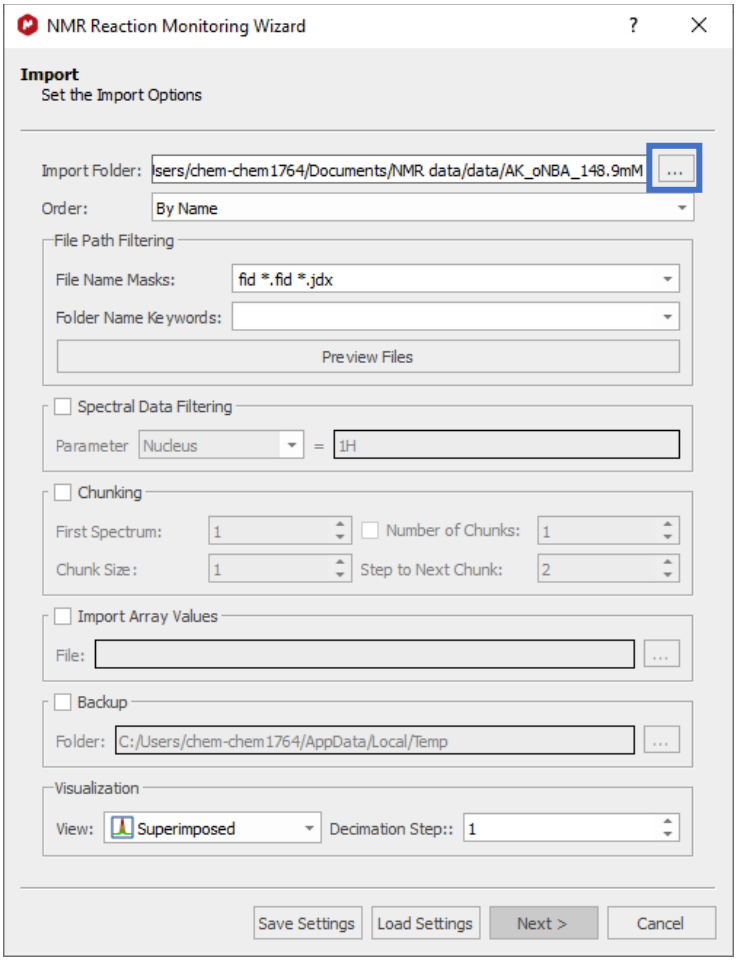
Under tools, select Reaction Monitoring, then Wizard. A pop-up window will appear.



Import the folder where the kinetic data is stored using the '…' button (blue box shown below). Ensure the data directory is correct by selecting 'Preview Files'. If correct, the file paths for all data will be shown.

(Acquisition Method 2 only) If multiple nuclei spectra were acquired, these must be processed separately. Tick the 'Spectral Data filtering' box and type the nuclei you wish to processed, i.e., '1H', '19F' or '31P'. This example data is only ^{1}H experiments so spectra data filtering was not selected.

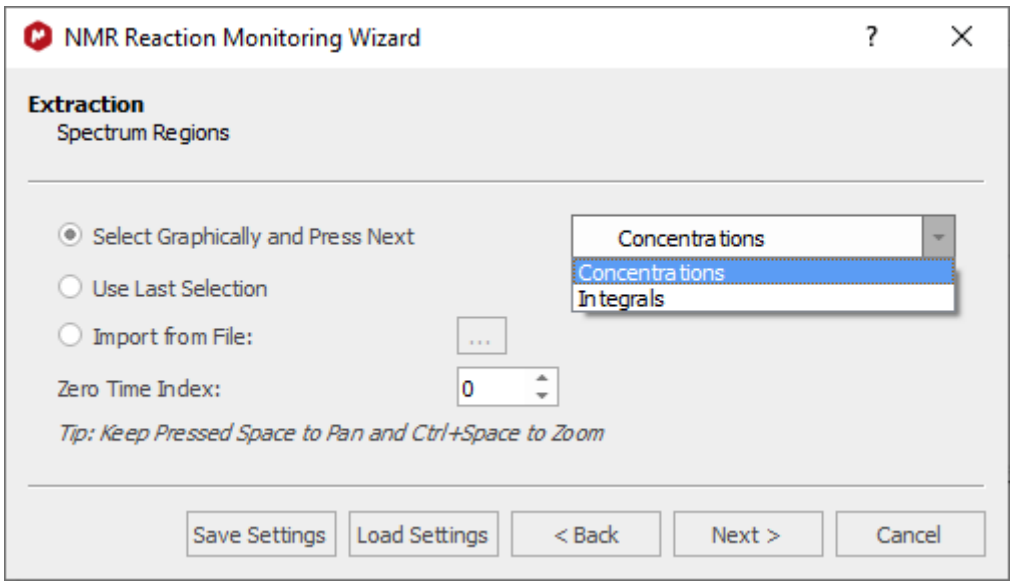
Under Visualization, select either stacked or superimposed, depending on how you wish to view the data. If you have a high number of spectra, superimposed is recommended to ease visualisation of the data. Select Next.



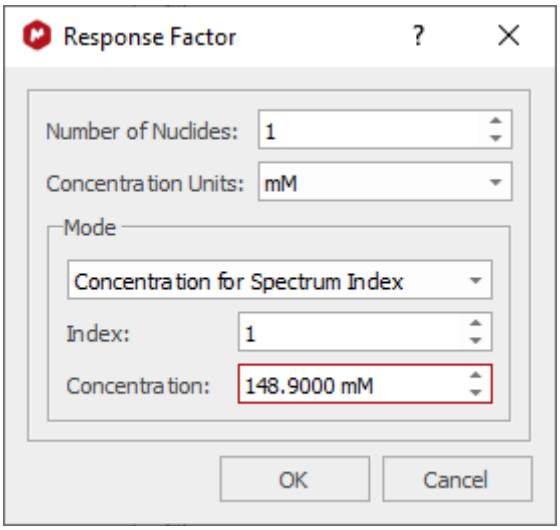
For processing, '*Default Mnova processing*' is typically sufficient. Click *Next* and the Wizard will Fourier Transform and phase all spectra. It is highly recommended to check the phasing and baseline of the spectra as this will affect the kinetic results. If needed, this can be modified later. If there are experiments with no data (for example, if the experiments weren't stopped earlier than originally set up), it may fail at this step. Remove any experiments that have no data associated with them and try again.

The next window will offer two options to track the signals of interest; concentrations or integrals. If the starting concentration of the SM is known in the first time point (spectra 1), select concentrations. If the reaction has started and the starting concentration of the SM is not exactly known, select 'Integrals'. Select the relevant option and move your cursor over to the spectra to integrate the SM signal.

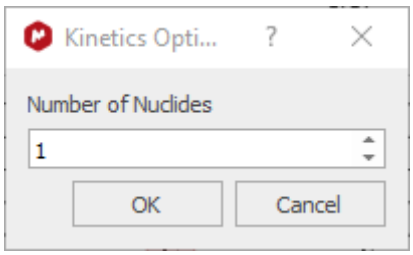
Hold the space key button to pan over the spectra and Ctrl+Space to open the zoom function. Release any keys to go back to the integrate function. You must integrate the regions of interest before you click next otherwise the procedure will have to be restarted.



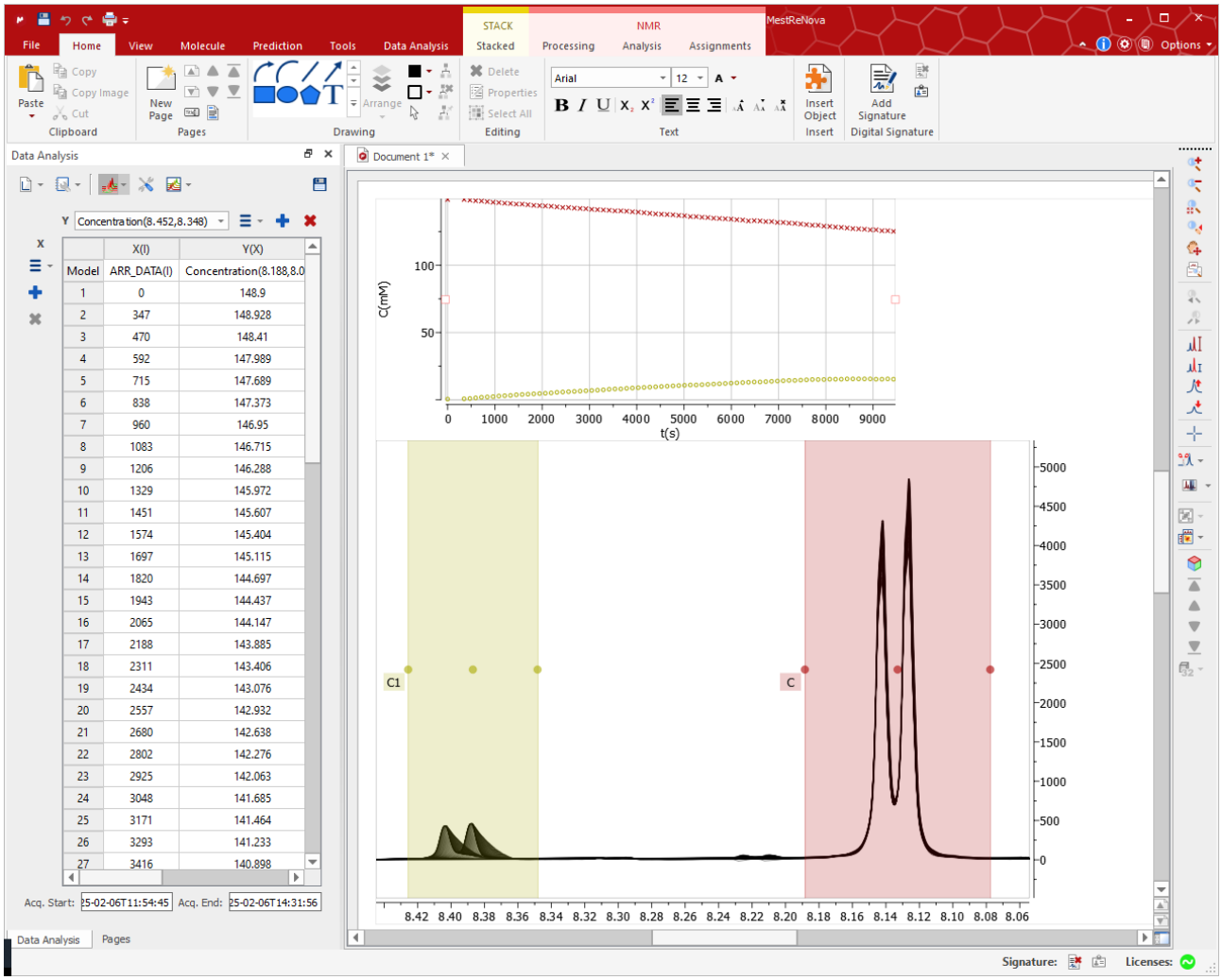
Once the SM signal is integrated, a response factor pop-up window will appear. Update all relevant information. The below example is for integrating a CH peak of the SM material at 148.9 mM. Select OK since done and the wizard will plot the integral of the SM peak as a function of concentration vs time. The time information has been directly extracted from the raw data. Check that this is correct. The Wizard will extract and plot all integrals of the SM on a graph where the y-axis is C (concentration in the specified units). The Wizard will also extract the time points for all data (x-axis).



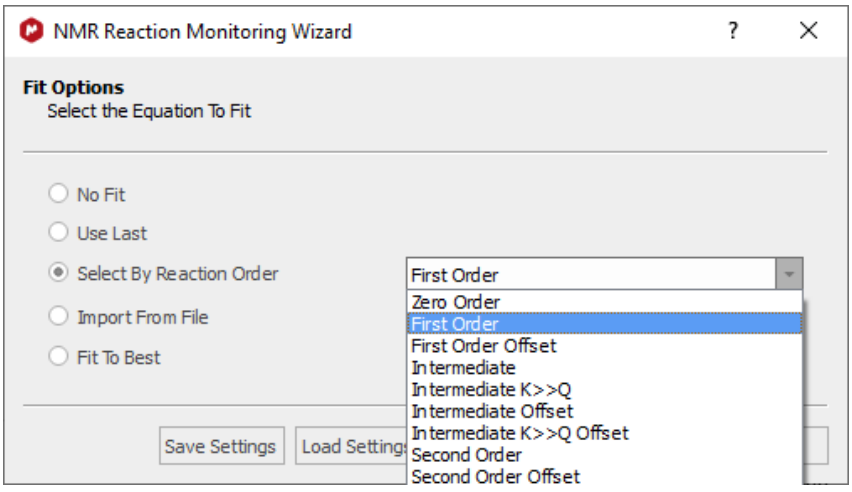
Integrate product signals of interest. A pop-up window will appear after integrating each one allowing you to specify how many nuclides correspond to the peak. Update if required then select OK.



The Wizard will plot all integrals of the product/s using the defined settings.



Click Next on the NMR Reaction Monitoring wizard.



MestreNova has the option to plot your data against multiple kinetic reaction order equations. If the reaction order is known, select the reaction order. It will then fit the data to this equation and give you the option to save the report (PDF) and MNova file data. If required, locate the file location you wish to save this and give the files a name. Click Finish.

To extract the data, click on the corner cell of the table (shown below in red) and select the notepad icon (green). Click on 'Report Table To CSV'' and save the data.