Dynamics Center Tutorial:
Kinetics

This tutorial contains a selection of spectra to be used in the exercises.

These data are confidential and must not be further distributed.

To run the tutorial, copy the folder tutorial_kinetics to your disk, e.g. into c:\

The following files then become available:

c:\tutorial_kinetics

ReactionA series of 1D kinetics spectra

tutorial_kinetics.pdf this document

Contact:

Dr. Anna Codina	+44 (0)24 7685 5200	Anna.Codina@bruker.co.uk
Dr. Klaus-Peter Neidig	+49 721 5161 6447	Peter.Neidig@bruker-biospin.de
Dr. Klaus Zick	+49 721 5161 6135	Klaus.Zick@bruker-biospin.de

The following assumes that the Dynamics Center software is installed and can be started, i.e. at least a FlexLM TOPSPIN_1D license is contained in the license file.
Novice users of the Dynamics Center should first consider looking at the tutorial named **General Dynamics Tutorial**.

Last updated:

April 30, 2013

Exercise 1:

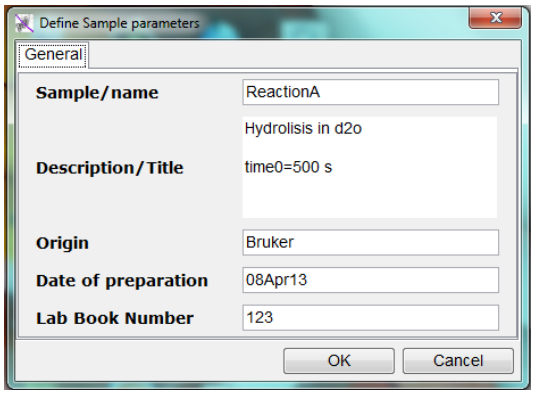
Acetic Anhydride Hydrolysis

This exercise illustrates how to obtain kinetic information from NMR data, using the acetic anhydride hydrolysis as an example[1]. The reaction was run in an NMR tube and followed by 1D ^{1}H NMR (series of 1Ds).

$$Ac_2O + H_2O \rightarrow 2 \times AcOH$$

Expand the Kinetics Method by clicking on the '+' icon, in front of **Kinetics**. Follow the steps from top to bottom.

1. Sample:



Start a new project by clicking on **Sample** and filling the various fields with the appropriate metadata.

2. Data:

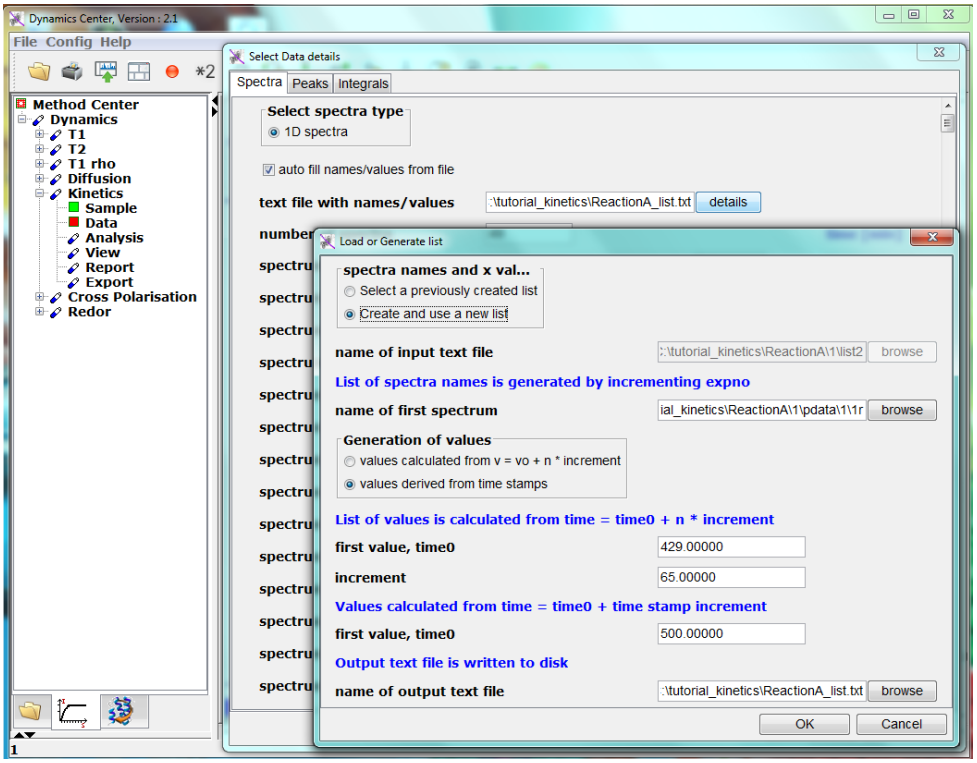
The **Data** menu is used to open the time dependent data and to choose analysis settings, such as peak picking and whether we want to work with peak intensities or integrals.

In this exercise we will open the data and use the time stamp to associate a time value to each spectrum. Since the reaction was run in a tube, there is a delay between mixing the reagents and the start of the reaction (time0), which will be taken into consideration.

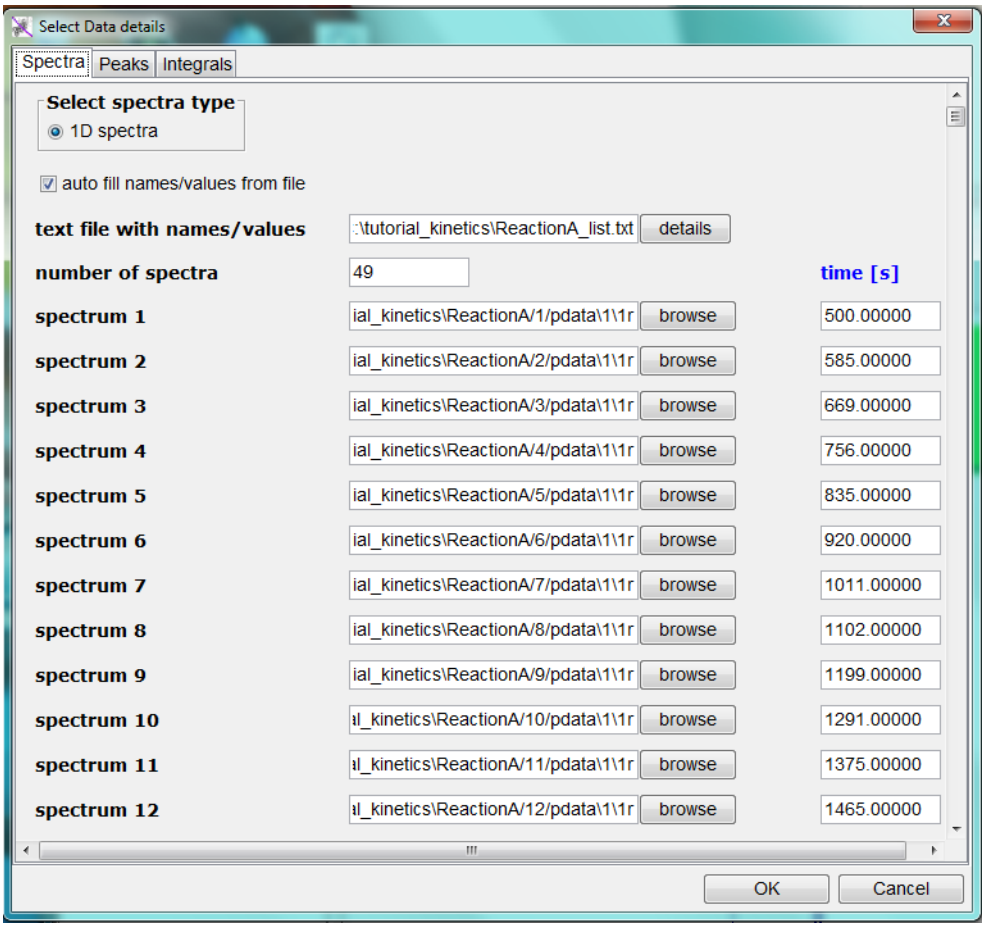
After clicking on **Data**, select **details** under the **Spectra** tab. This opens a pop up menu that enables us to create a list. Select **create and use a new list** and browse to the 1r file of the first spectrum, C:\tutorial_kinetics\ReactionA\1\pdata\1\1r.

Select **values derived from time stamps** and set the time 0 to 500 (seconds).
Set the output path for the list by choosing a folder name and a name for the text file, e.g. C:\tutorial_kinetics\ReactionA_list.txt. Folders not yet existing will be created.

[1] Susanne F., Smith D., Codina A., *'Kinetic understanding using NMR reaction profiling'*, **Org Process Res Dev**., 16, 61 (2012)

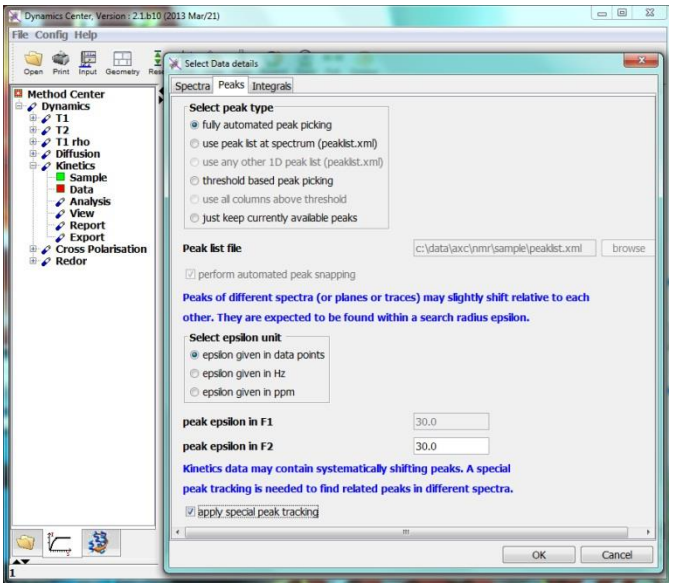


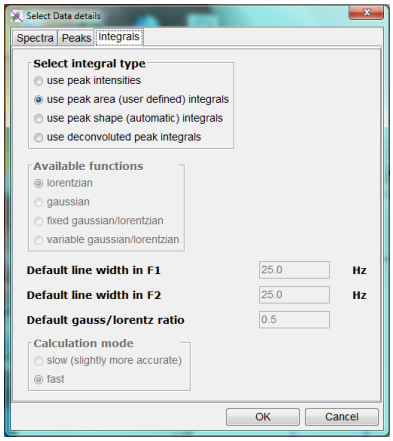
Press 'OK'. The **Spectra** tab will be auto-populated from the newly created list as shown below:



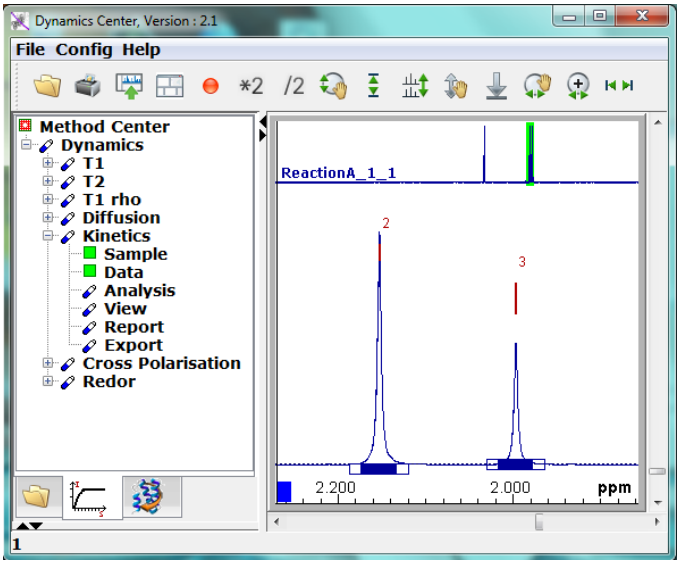
Under the **Peaks** tab, select **fully automated peak picking** and **apply special peak tracking**. Set **peak epsilon in F2** to 30 points, appropriate for the chemical shifts differences seen in this reaction.

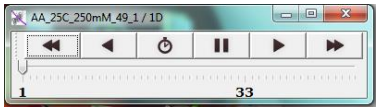
Select **use peak area (user defined) integrals** under the **Integral** tab as shown below.





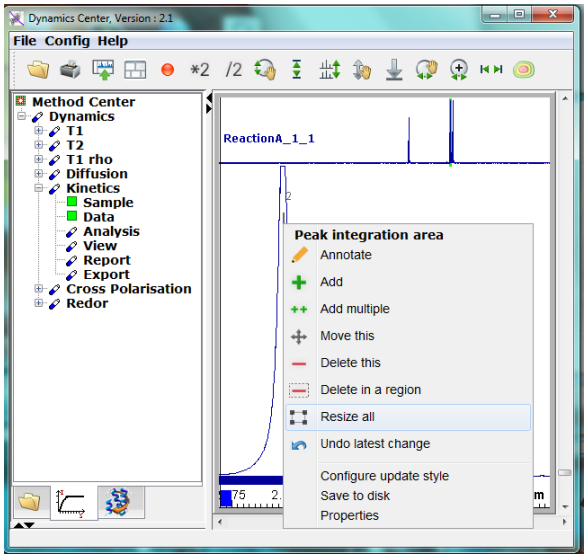
After clicking 'OK', the first spectrum will be shown on the screen. Use the slider to check the peak picking. The peaks for acetic anhydride and acetic acid have been correctly picked despite the significant signals shift occurred during the reaction. This is because of the **automatic peak tracking**.





The slider (top) allows the visualisation of the individual spectra in the dataset. The sequence of spectra can also be played as a 'video' by selecting the double arrows.

For each picked peak, integration regions have also been defined automatically, with an initial default width of 0.07 ppm. The integration regions can be modified by the user.



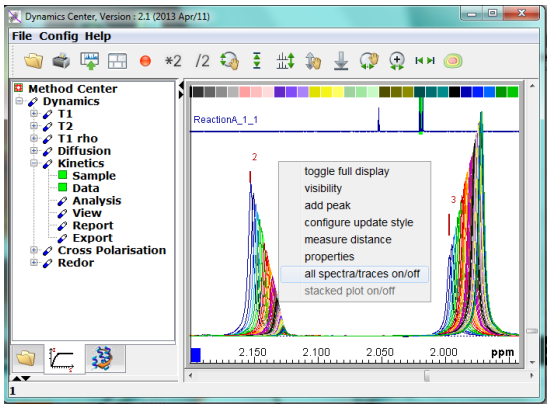
*The selection of option **use peak area (user defined) integrals** enables post-analysis manual modification of the integrals.*

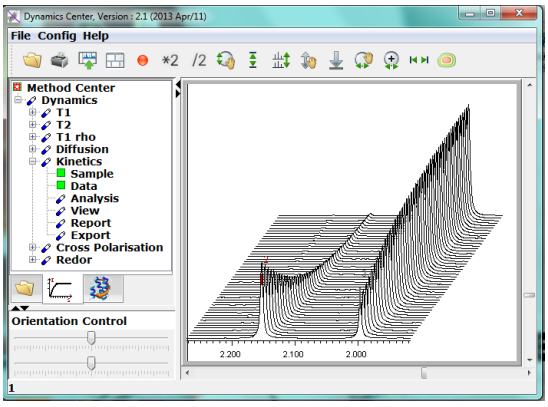
Integral regions can be moved by clicking and dragging the integral bar. A hand is shown when the cursor is on the bar. Integrals can be resized by clicking and dragging the edges of the bar. An arrow will be shown when the cursor is placed on the edges of the integral bar.

*More **Peak integration area** options are offered, in a pop up menu, by right clicking either on the peak or the integral bar.*

Right click on one of the integral bars and select **resize all**. Set the **region width** to 0.1 ppm to make all the integration regions slightly wider.

Right click on an empty space of the spectra to visualise all the traces at once and to generate a stacked plot.





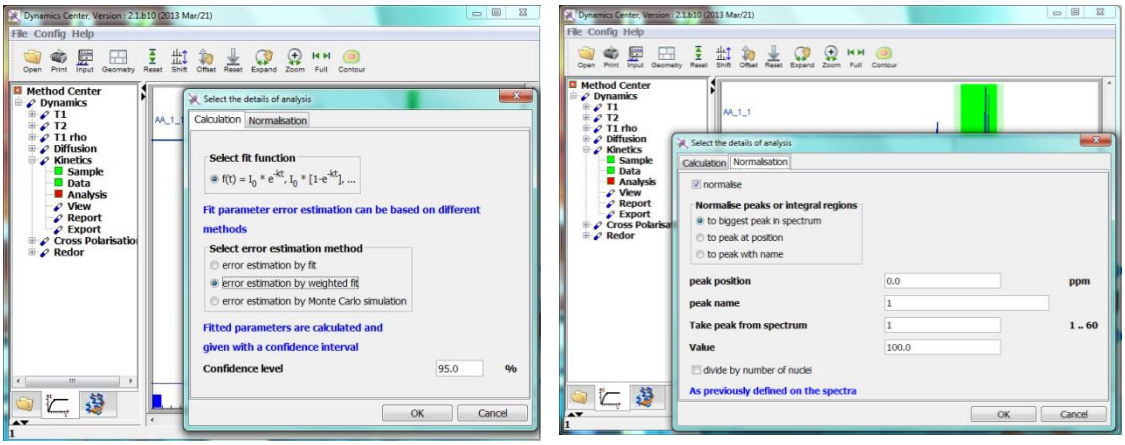
The Orientation Control sliders allow changing the perspective and height of the stacked plot.

Switch the stacked plot off (right click on an empty space and select **all stacked plot on/off**) to go back to single spectrum view.

One may save the status of the analysis to a project file at any time, by right clicking on **Kinetics** and selecting **Save as**. Save with a suitable name in a directory of your choice, e.g. C:\kinetics_tutorial.project.

3. Analysis

In the Analysis menu you will find the tools to represent kinetic profiles and to do curve fitting. The fitting step is automatic, the user does not need to enter a fitting function or starting parameters. The program tries to fit the experimental data to 12 different fitting functions. The function that best describes the experimental data will be selected automatically and reported. There are different ways to calculate the error for each fitted parameter. Click on **Analysis** and select **error estimation by weighted fit** from the **Calculation** tab. This is recommended for kinetics data.

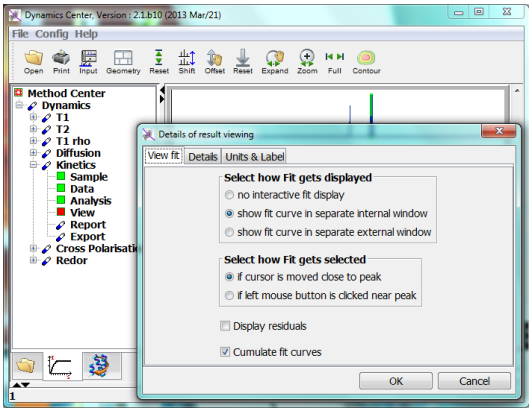


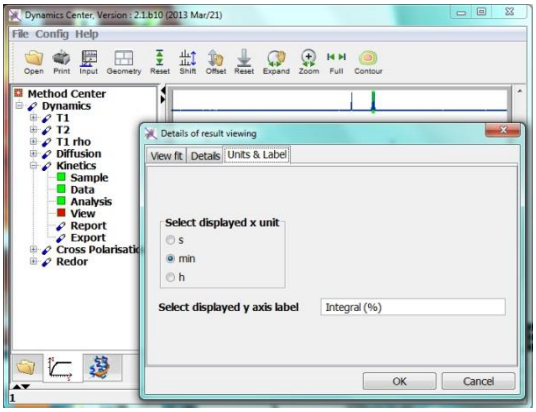
The integral values extracted from the NMR data can be normalised using different methods. Click on the **Normalisation** tab and select for example **normalise** integral regions **to biggest peak in spectrum**. Set **Take peak from spectrum** to 1 and **Value** to 100. Click 'OK'. This will normalise the integral values based on the biggest integral in spectrum 1 being 100. The calculation time needed for the complete analysis depends on the number of spectra and peaks. In the example here it will be finished within moments and **Analysis** will turn green.

4. View:

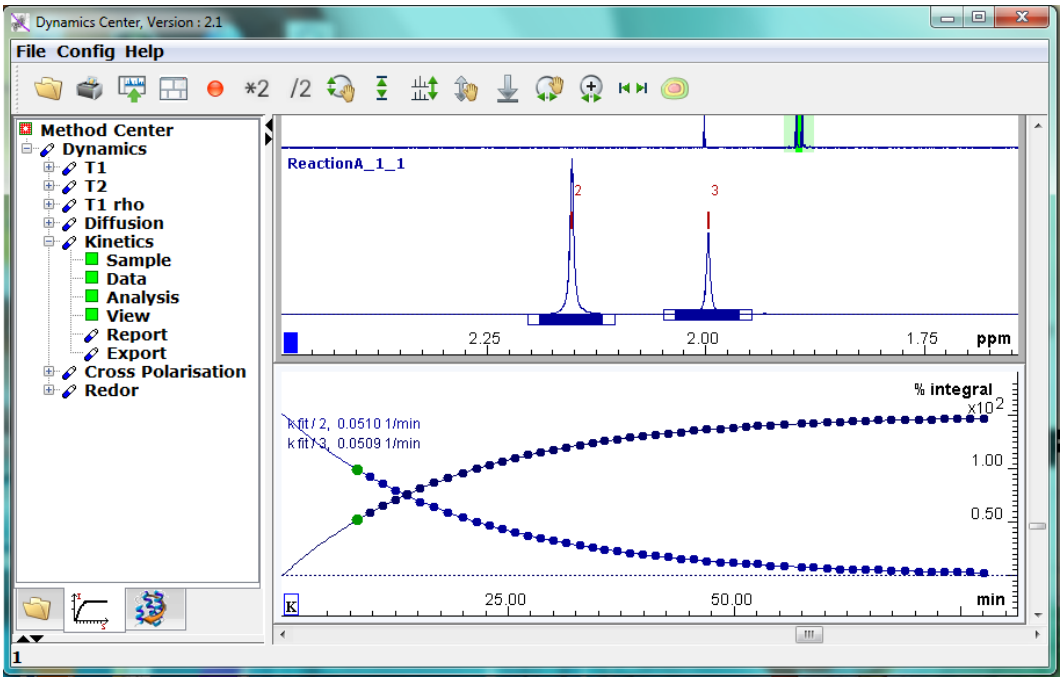
Under the **View** menu, we will find different possibilities to visualise our data. Once in the **View fit** tab, select **show fit curve in separate internal window**; **if cursor is moved close to peak** and **cumulative fit curve**. This will allow us to get fit curve displays by moving the cursor to individual peaks. The individual curves will all be cumulated and shown in overlay for better comparison.

To improve the fit curve display, click on **Units & Label** to choose the units and labels of the axis. Select 'min' and set the label of the Y axis as 'Integral (%)'. Click 'OK'.





When the cursor is moved over the peaks, the profiles Integral *vs.* time, shown below, are obtained.



For better selection of peaks you may customise the spectrum display by zooming into smaller regions. The fit curve display keeps track of what has already been selected to avoid multiple displays of the same curves. You can also move the cursor into the fit curve window and customize the display (e.g. scale, zoom). A right mouse button click offers a pop up menu with some additional options. In order to change the selection style of the fit curves, or to choose other display options, click on **View** again.

5. Report

Use the **Report** menu to generate a PDF report with your results. Individual components of the report (spectrum, fit curves, result table) can be selected in a dialog window. Also, select

an output name in the directory of your choice, e.g. C:\tutorial_kinetics\report.pdf, and press 'OK'. The output default directory can be defined under **Configuration / Preferences** (top menu).

At this point you may save the status of the analysis. Right click on **Kinetics** and select **Save**. Work will be saved to the same project file as specified last time. To repeat the analysis at a later time, right click on **Kinetics** and use **Open** to reload the project.

Exercise 2:

Acetic Anhydride Hydrolysis - Part II

Exercise 1 illustrated how to analyse kinetics data with minimal human intervention. Exercise 2 shows a wider range of tools, some of which will be needed for the analysis of more complicated data.

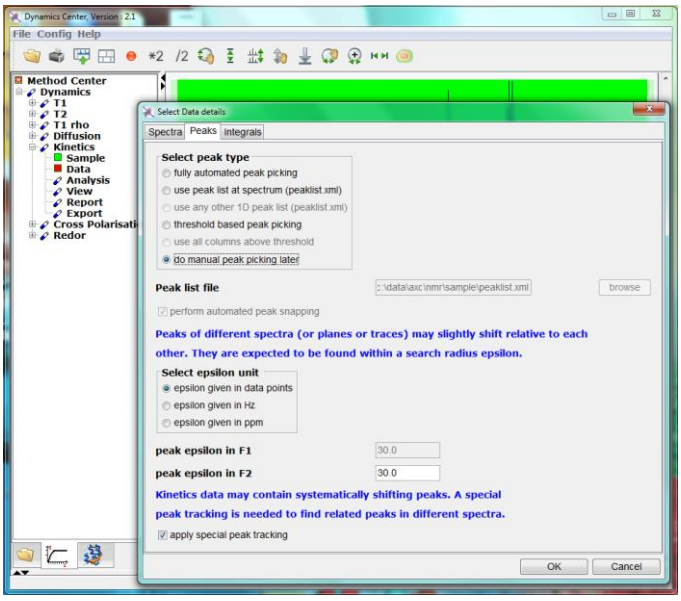
Start by opening the project we saved in the previous exercise. Right click on Kinetics and select **Open**. Browse to the directory where the project was stored (e.g. C:\kinetics_tutorial.project).

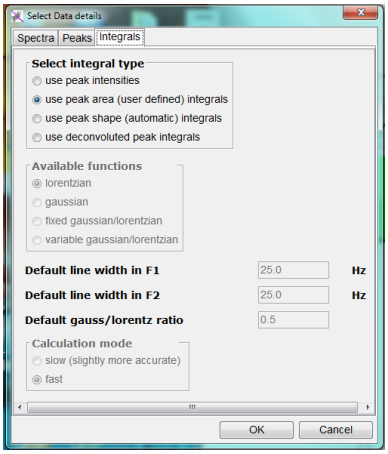
1. Sample

   The sample information is the same, so we will not modify it.

2. Data

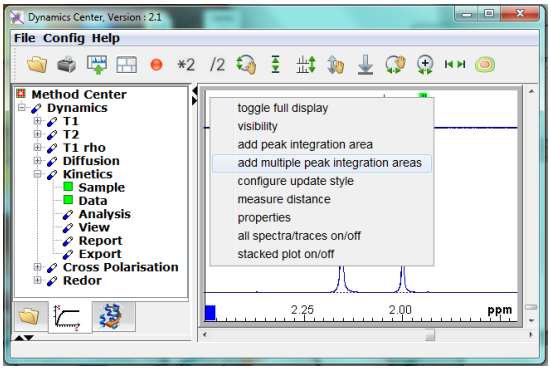
   We will re-analyse the ReactionA data. Click on **Data** and under the **Peaks** tab, select **do manual peak picking later** and **apply special peak tracking**. Leave the peak tracking settings as they are: **epsilon given in data points** and **peak epsilon in F2** = 30. Select **use peak area (user defined) integrals**, under the **Integrals** tab and then 'OK'.

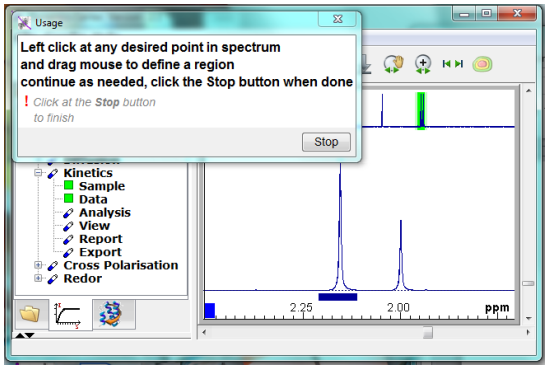




The option **do manual peak picking later** allows the user to do manual peak picking from scratch. In this case, the peaks and integral regions automatically determined in exercise 1 will be deleted.

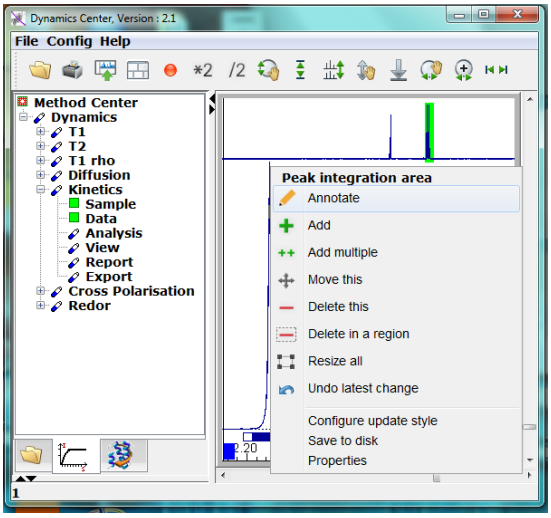
Expand the region of the spectrum that contains the two biggest peaks (~ 2.50 – 1.75 ppm) by clicking and dragging the cursor over the peaks. Right click on the spectrum and select **add multiple peak integration area**. Click and drag over each of the peaks to define the desired integral regions. Click 'Stop' when finished.

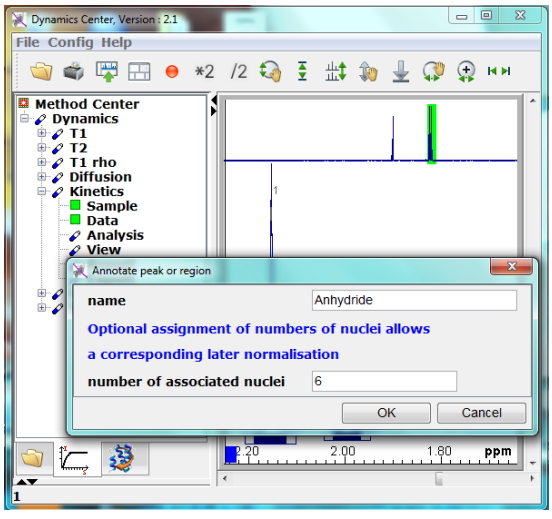




Use the slider to check that the integral regions have been correctly propagated to the rest of the spectra.

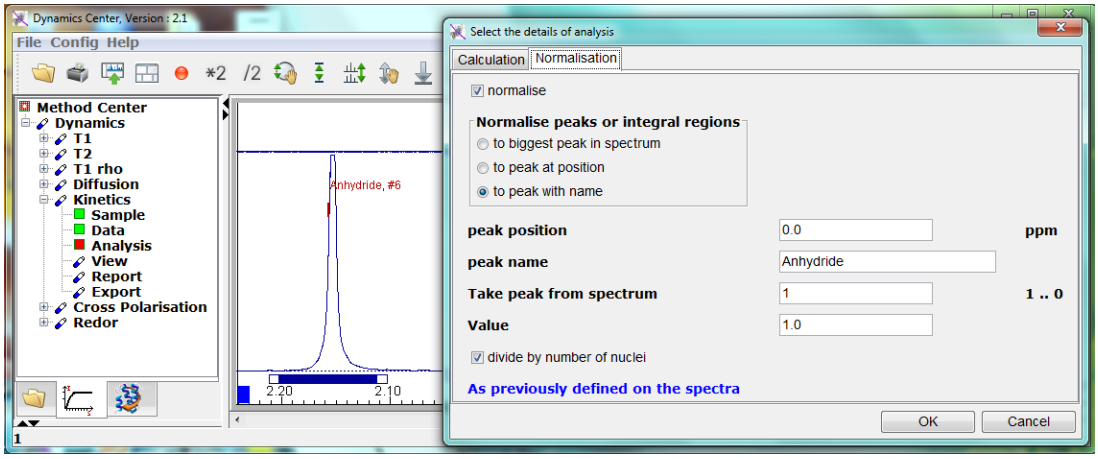
We will now label the peaks and set the number of protons that are under each peak, which will enable the representation of concentration and/or equivalents profiles later on. The peak at 2.153 ppm corresponds to the acetic anhydride (6 protons) and that at 1.999 ppm to acetic acid (3 protons). By right clicking either on the peaks or on the integral bars, a menu pops up. Select **Annotate**. Set the **name** and **number of associated nuclei** for each peak, as shown below. Press 'OK'.





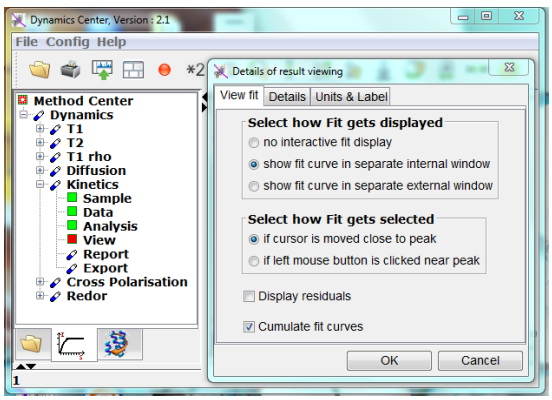
3. Analysis

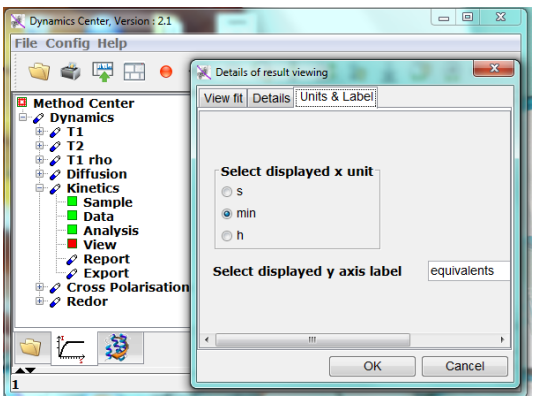
Click on **Analysis**. Leave the Calculation tab as it is (i.e. **error estimation by weighted fit**). Under the **Normalisation** tab, select **normalise** to **peak with name** and set the **peak name** as 'Anhydride'; **Take peak from spectrum** '1' and **Value** '1'. This will normalise the integral values based on the integral of the Anhydride peak in the first spectrum being 1. Select **divide by number of nuclei**.



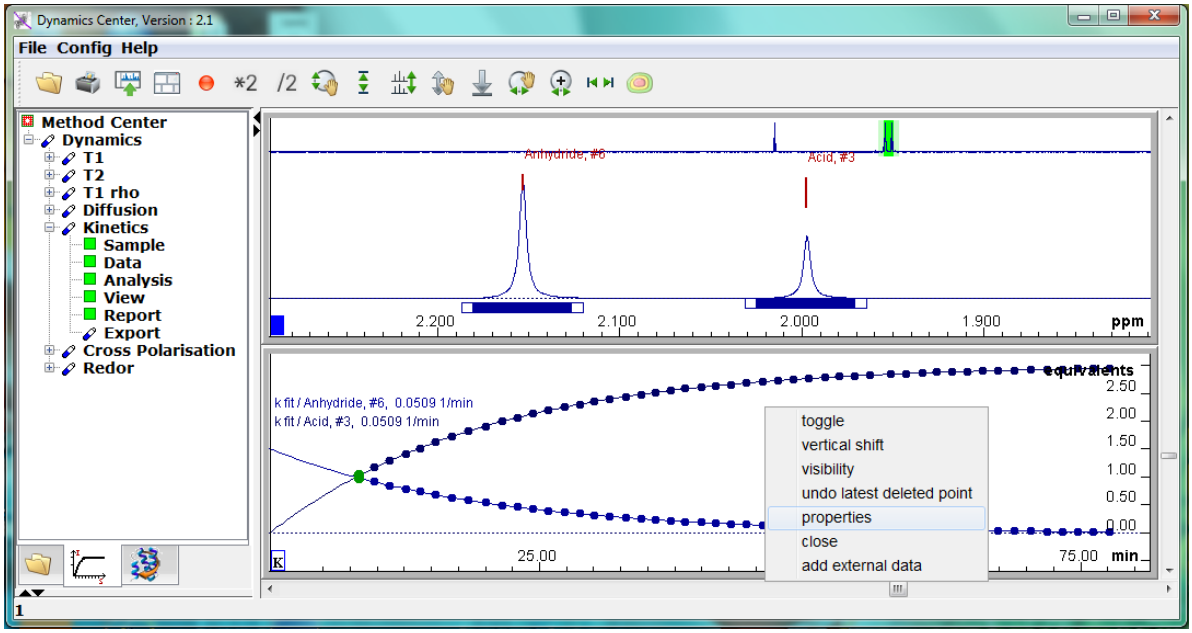
4. View

Click on **View**. Leave the **View fit** setting as they are: **show fit curve in separate internal window**; **if cursor is moved close to peak** and **Cumulative fit curves**. Under **Units & Label**, **Select displayed x unit** in 'min' and set **Select displayed y axis label** as 'equivalents'. Click 'OK'.

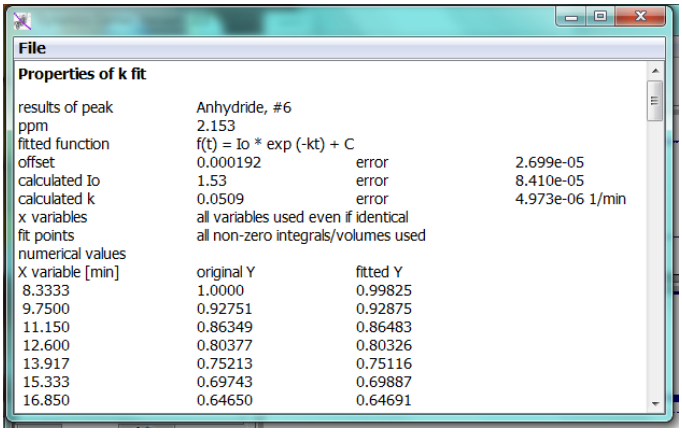




Move the cursor over the peaks to visualise the kinetics profiles equivalents *vs.* time (min). You should obtain the graphic shown below. Re-scale the fit curve display by turning the mouse wheel if needed. The obtained fitted rate constants may differ slightly from those in the figure, depending on the integral regions, which have been defined manually in this exercise.

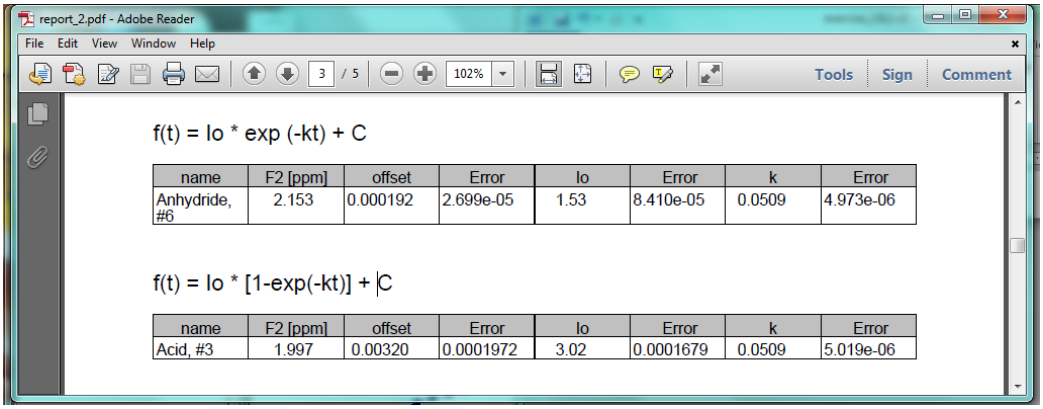


Right click on an empty space of the profiles window and select **properties** to see the details of the fitting.



5. Report

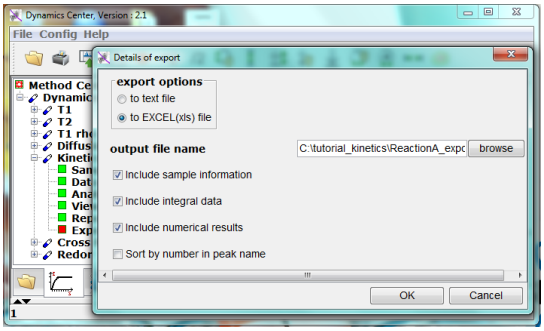
Click on **Report** and choose an output file name (e.g. C:\tutorial_kinetics\report_2.pdf). Tick all the boxes and select **include all fit curves**. The option **include all parameters** includes fitting functions and parameters in the report.

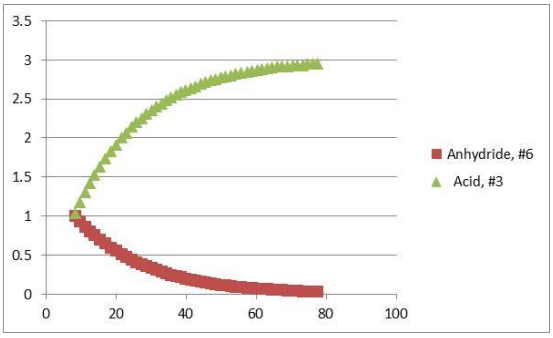


Right click on **Kinetics** and select **save as** to save the project with a different name (e.g. C:\kinetics_tutorial.project_2).

6. <u>Export</u>

Click on **Export**; select **export options** to **EXCEL (xls) file**; set an **output file name** (e.g. C:\tutorial_kinetics\ReactionA_export.xls) and select the following options: **include sample information**; **include integral data** and **include numerical results**. Click 'OK'.





Open the file ReactionA_export.xls in Excel. Copy the values under the Integrals tab. Paste them in a new sheet using **paste special / transpose**. **Hide** the second column (peak name); select the remaining columns and **insert** a **Scatter** type graphic. The graphic obtained should look like that above, depending on the Excel version used.