Dynamics Center Tutorial:
Protein Dynamics

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 just copy the folder tutorial_pdc to your disk, e.g. into c:\

The following data are then available:

c:\tutorial_pdc

2D-Ref	2D hsqc spectrum with a good peak list
185-NOE	pseudo 3D data for NOE analysis
1065- T1	pseudo 3D data for T1 analysis
1066-T2	pseudo 3D data for T2 analysis
ubi_fasta.txt	fasta file of ubiquitine, contains amino acid sequence
1UBQ.pdb	ubiquitine pdb file (no protons)
1UBQ_H.pdb	ubiquitine pdb file with protons

tutorial_pdc.pdf this document

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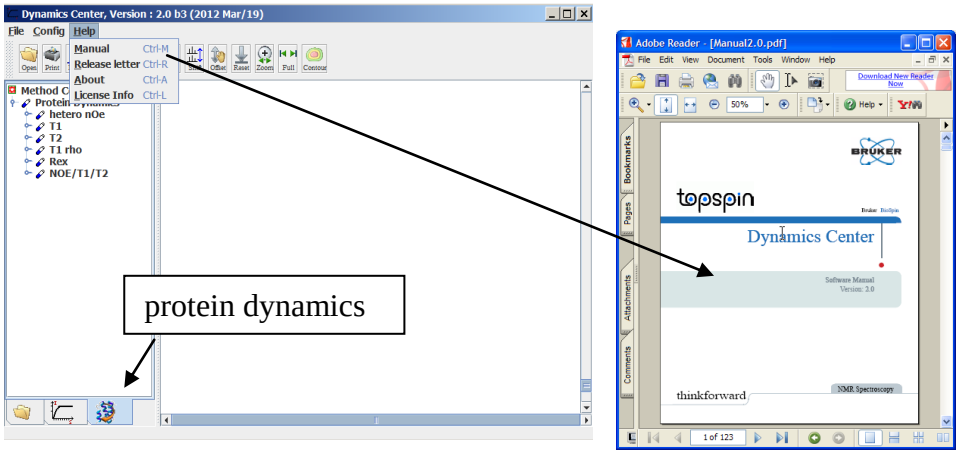
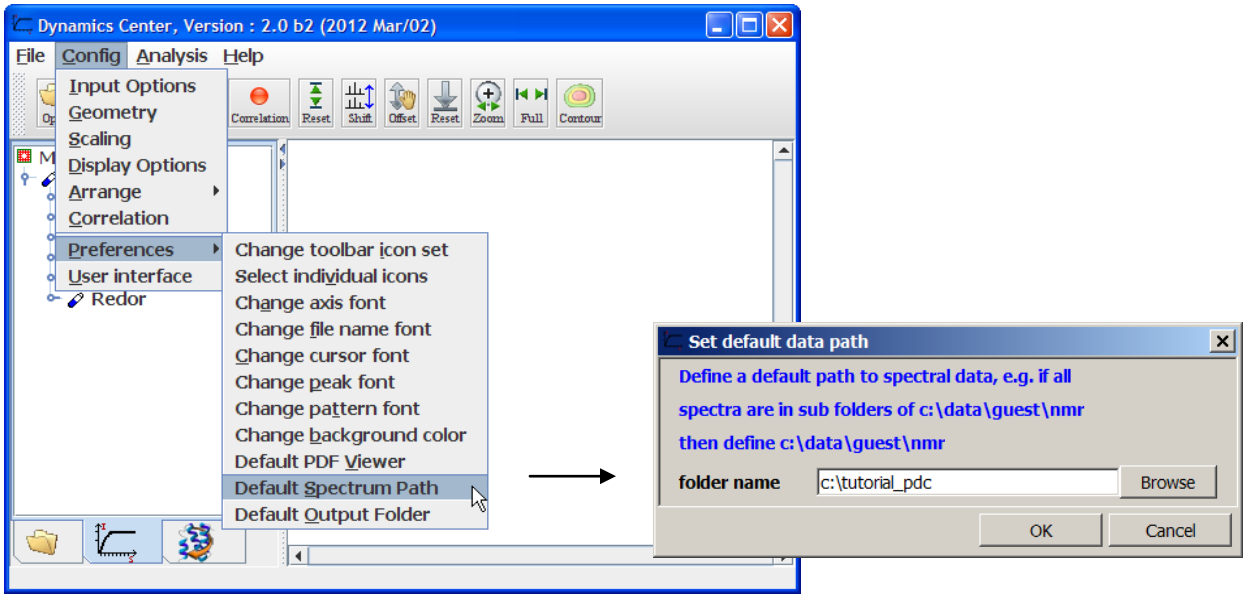
Exercise-1: General Features

- Start the Dynamics Center, use **Help/Manual** to check the available hand book.



There are three tabs in the lower left area. One is to display a file system explorer, one is for general dynamics and one for protein dynamics. This tutorial uses protein dynamics. By clicking on it a method tree containing hetero nOe, $\mathbf{T_1}$, $\mathbf{T_2}$, $\mathbf{T_{1rho}}$, **Rex**, and **NOE/T1/T2** is shown.

- Using **Config/Preferences** to customize the DC

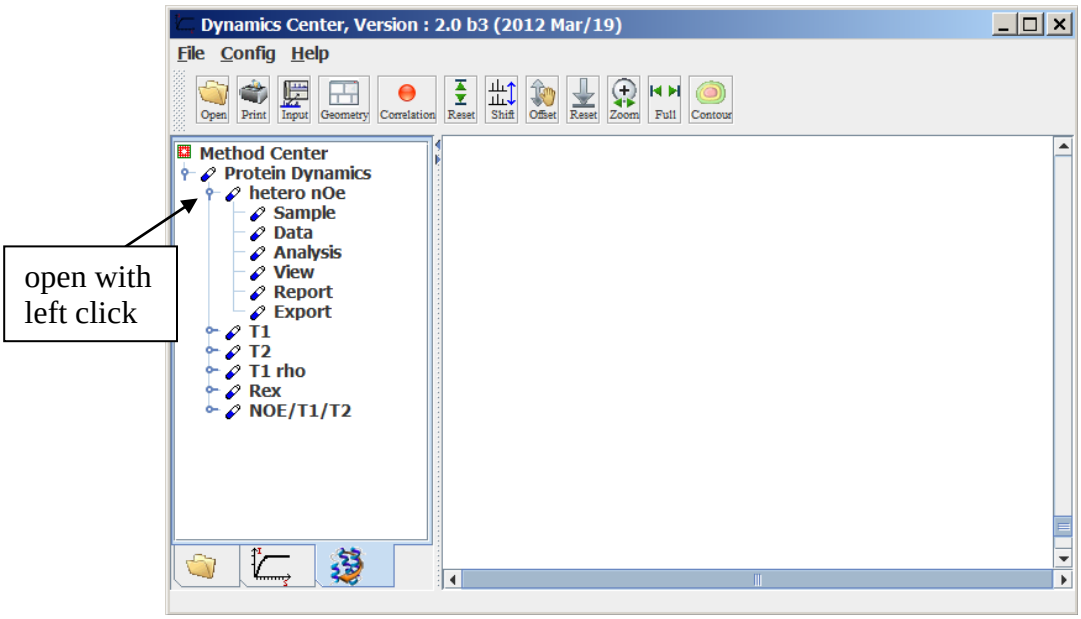


The data analysis is completely performed by activating methods on the method tree. The main menu bar only serves for some global purposes, especially setting

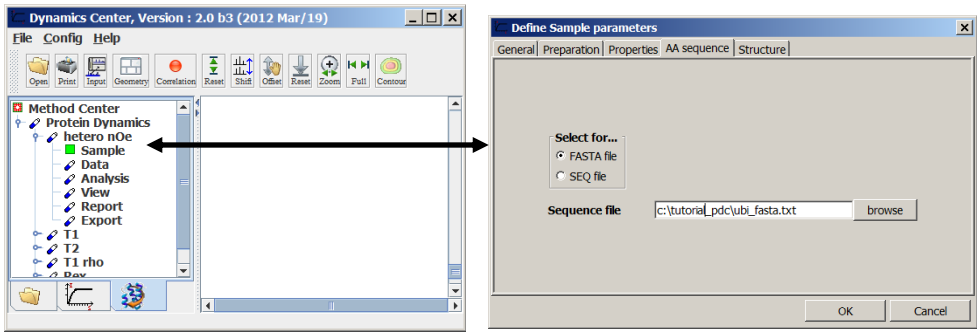
preferences. Via Config/Preferences/Default Spectrum Path one can for example set the starting point for spectra searches. All the spectra used in this tutorial are for example in further sub-folders of c:\tutorial_pdc, thus setting the default spectrum path to c:\tutorial_pdc would be appropriate.

Exercise-2: hetero nOe Method

- Protein Dynamics should already be opened on the method tree, thus we can **left click** to the hetero nOe method to open it as well.

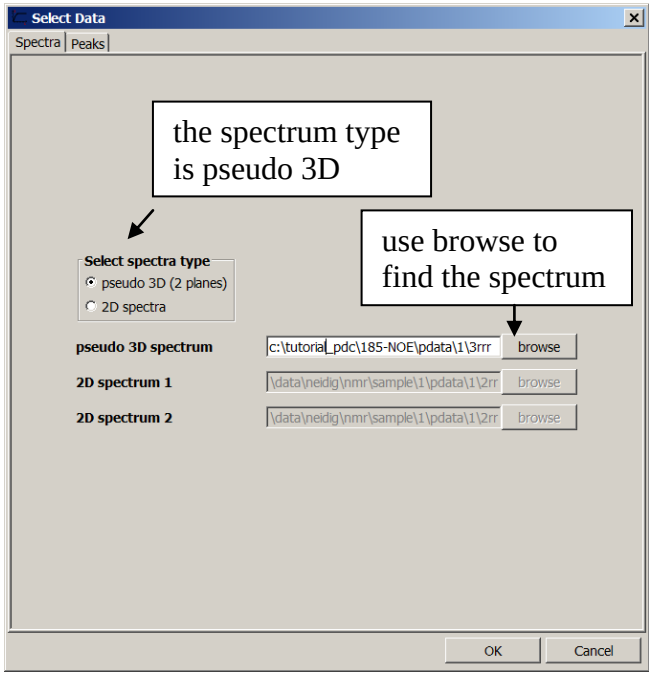


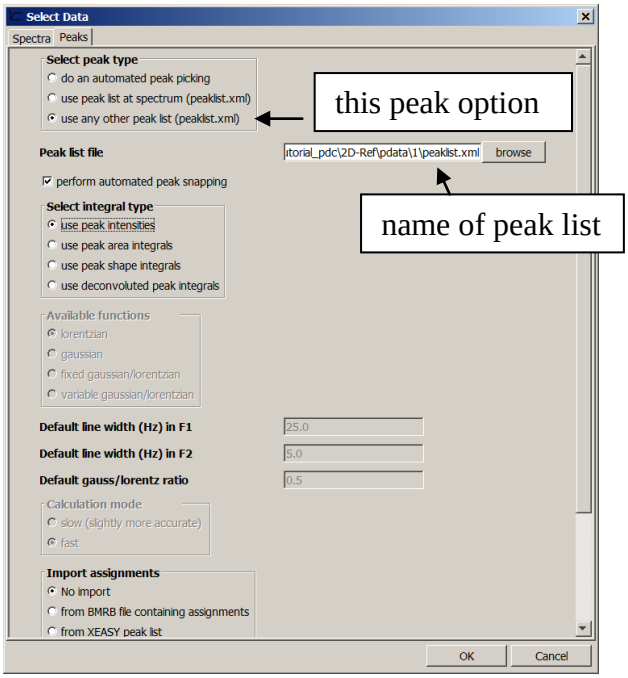
- At first we select **Sample** to define some parameters, especially the amino acid sequence. Sample gets a **red** marker and a dialog window opens.



Select the **AA sequence** tab in the dialog window and specify **FASTA** format and **c:\tutorial_pdc\ubi_fasta.txt** for the amino acid sequence. As a structure file select **c:\tutorial_pdc\1UBQ_H.pdb** When closing the dialog window with OK, the Sample leaf turns **green**.

- Now select **Data**. In the dialog window define **c:\tutorial_pdc\185-NOE\pdata\1\3rrr** as a **pseudo 3D** spectrum. Then select the **Peaks** tab. There is a good 2D HSQC spectrum available and we want to use the peak list of it. Therefore we choose **use any other peak list** and provide the name of the peak list as: As peaks take **c:\tutorial_pdc\2D-Ref\pdata\1\peaklist.xml**. It is advised





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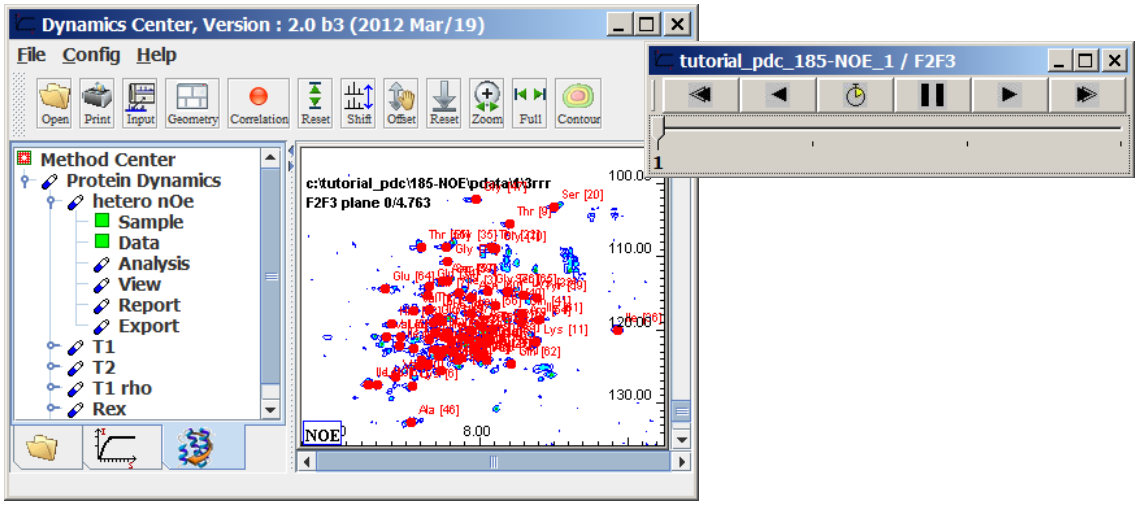
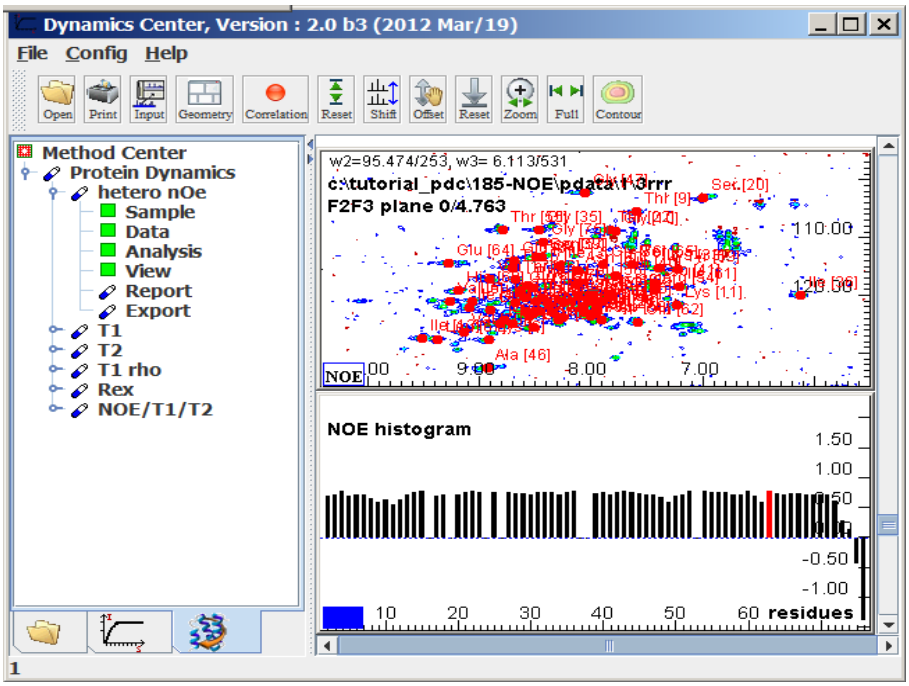
to also select **peak snapping** because the peak positions in the 2D spectrum might deviate a bit from the those in the pseudo 3D spectrum.
As integration select **intensities**. When searching for corresponding peaks in different planes a search radius is applied. 3 data points in each dimension is appropriate.

After clicking OK in the dialog window, **Data** gets **green** and spectrum and peaks get loaded. Using the data panel you can navigate to other planes if needed.



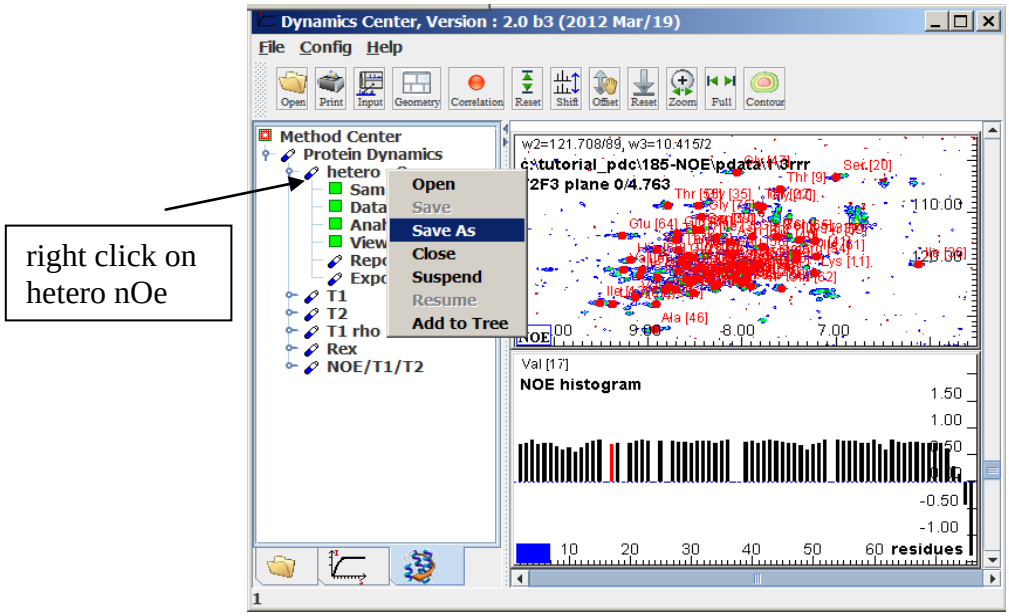
- **Analysis** is the next item to execute. The hetero nOe method does not need any settings of analysis parameters and runs automatically. After a moment you get the message that all NOE integral ratios have been calculated.

- **View** is the next item to execute. It does not need any settings and the display is generated automatically.



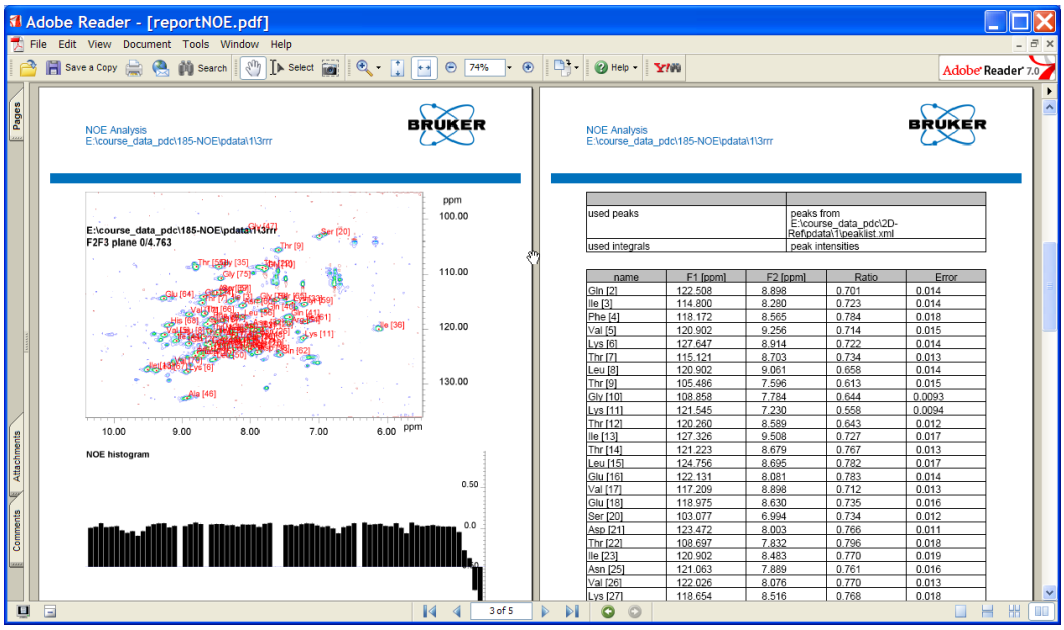
You should see spectrum and histogram (NOE ratios vs. amino acid sequence). Now play around a bit: move the cursor, zoom the histogram, double left click at the zoomed histogram, right mouse button click at histogram and properties from the popup menu etc.

- In order to save the current status do a **right mouse click** at the hetero nOe method and select **Save As**.



As a project file you can specify an arbitrary name, for example **c:\tutorial_pdc\NOE.project**. The extension .project is advised.

- Finally, execute **Report** on the method tree. In the up coming dialog window select any options you want, for example all. As an output name for the pdf file specify anything, e.g. c:\course_data_pdc\reportNOE.pdf. AcroRead is launched and you should get a display like

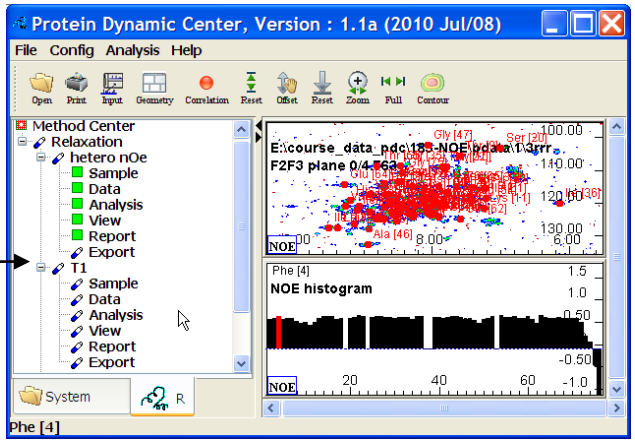


If there is no Acroread on your system you can use **Config/Preferences/Default PDF Viewer** to select another pdf viewer.

Exercise-3: T1 Method

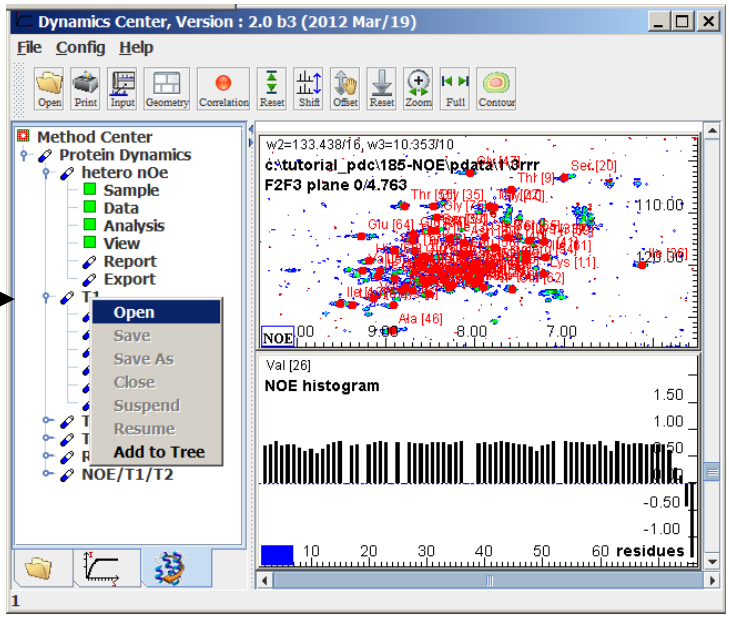
- Leave the display as is. If not yet open, open the T1 method with a left click at T1 on the method tree.

left click on T1



- Normally, we would now execute **Sample**, **Data** etc. as in exercise-2. But the data we have, come from the same sample. Therefore, a lot of information is identical to what we have specified in the hetero nOe method which we have saved to a project file. Therefore we just load it to the T1 method by doing a **right click** to T1 and selecting **Open**.

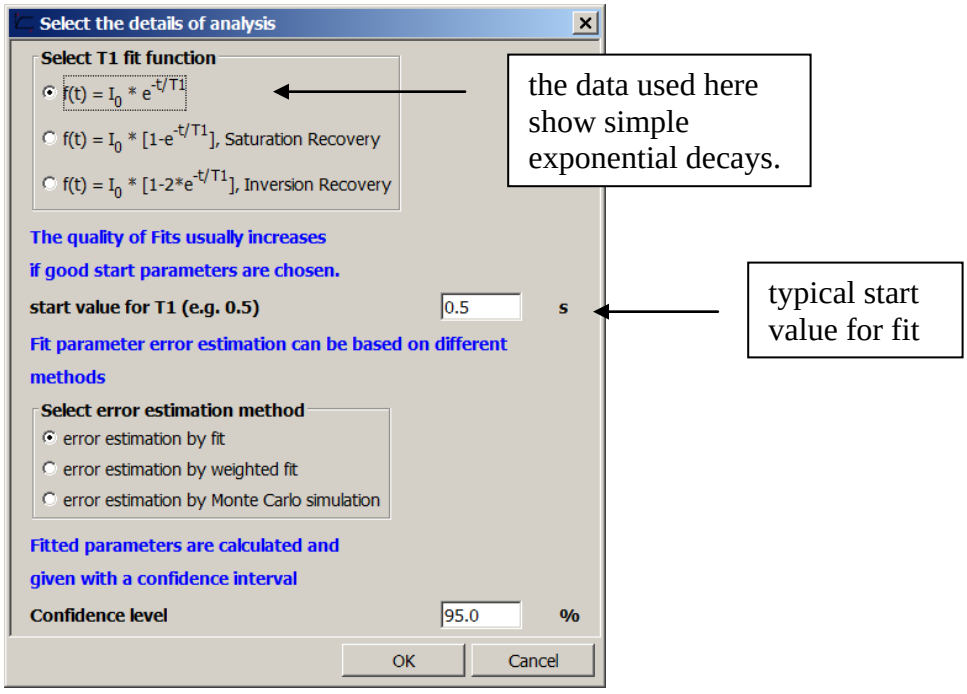
right click on T1 to get popup



As a project file name we specify **c:\tutorial_pdc\NOE.project**. It gets loaded and **Sample** on the method tree gets already green.

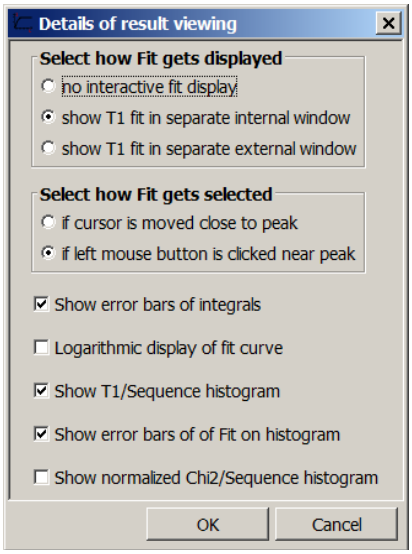
- We can directly continue with **Data**. The only thing which has to be changed in there is the spectrum name. It is **c:\tutorial_pdc\1065-T1\pdata\1\3rrr**. Either type it or use the browse button. There is another new tab called **Lists** in the dialog window. There we can for example specify how to handle multiply measured mixing times. Just take the default settings.

- Next, activate **Analysis** on the method tree. This time Analysis is not automated, it needs some settings. A dialog window is coming up.

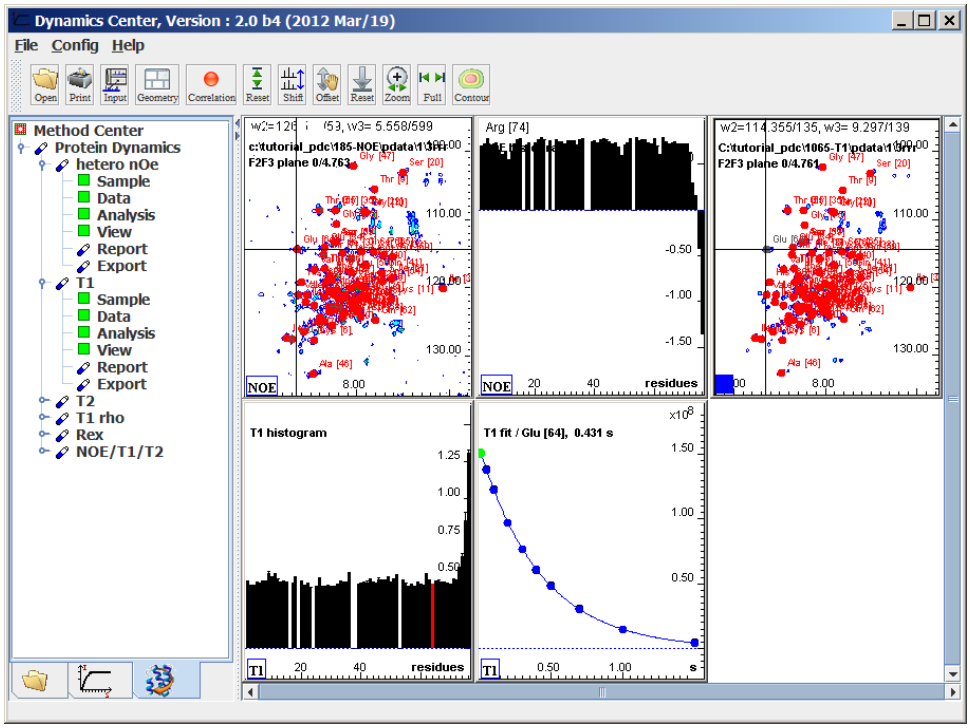


The spectrum used measures **exponential decays** which is the first option. As a starting value for the T1 fit chose **0.5**. For fit error estimation chose the second option. It evaluates signal-to-noise and variance of the repetition experiments. A typical confidence interval is **95%**. The curve fitting is quite fast and finished after a few moments.

- By clicking at **View** we can customize the display of the results in a dialog window. At first we select to display various fit curves in a window contained in the main Dynamics Center window. We furthermore chose to get a fit curve display whenever we do a left click to a peak. The other alternative would have been that we just move the cursor to a peak (without click). However, this makes it then difficult to move the cursor out of the spectrum window without coming close to another peak.

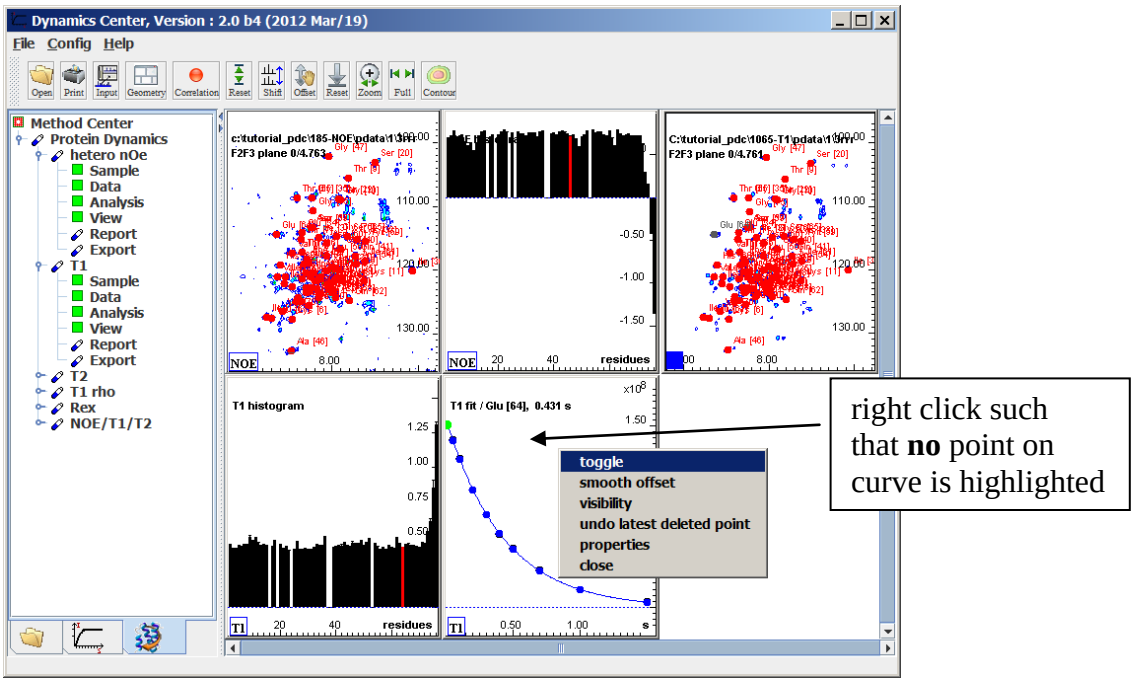


Select other options as shown, i.e. error bars of integrals switched on, T1 histogram switched on and error bars shown on histogram. The resulting display should look as follows if you left click to a peak in the T1 spectrum.

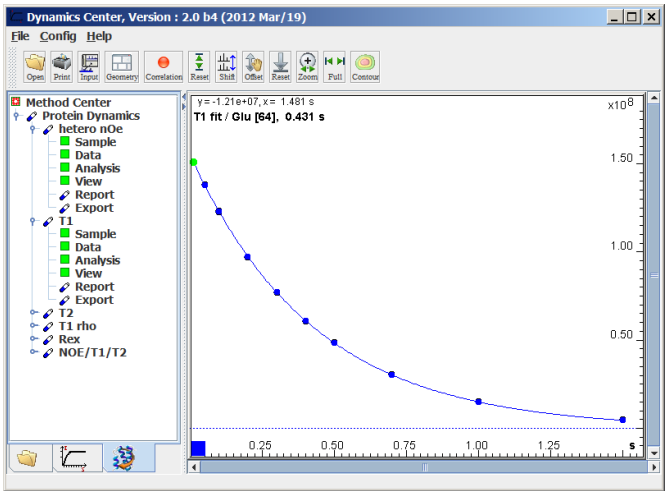


Every time you move the cursor to another peak and click the left button, the T1 fit curve updates. Also see the corresponding highlighted residues in the histogram. All objects on the display know each other. The cursor is a multiple linked cursor.

- Play around a bit. The windows became quite small. Suppose you want to see the current fit curve in more detail. Then **right click** at it but such that you are not near a fit point (no fit point should be highlighted with a red color). From the popup menu select **Toggle**.

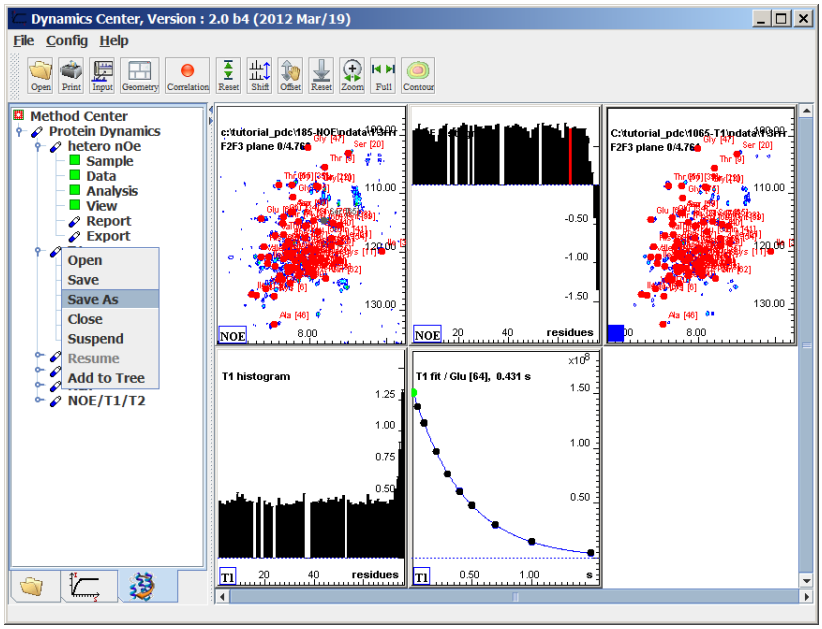


Get a display like:



- All display objects except the fit curve disappeared. To get back to the original display just do a right click at the fit curve (no fit point highlighted in red) and chose **Toggle** again.

- Now it is time to save the T1 method to a project file as well. Right click on T1 and select **Save As** from the popup menu.



It is important to select **Save As** since we want to save the T1 project to a different file, e.g. **c:\tutorial_pdc\T1.project**.

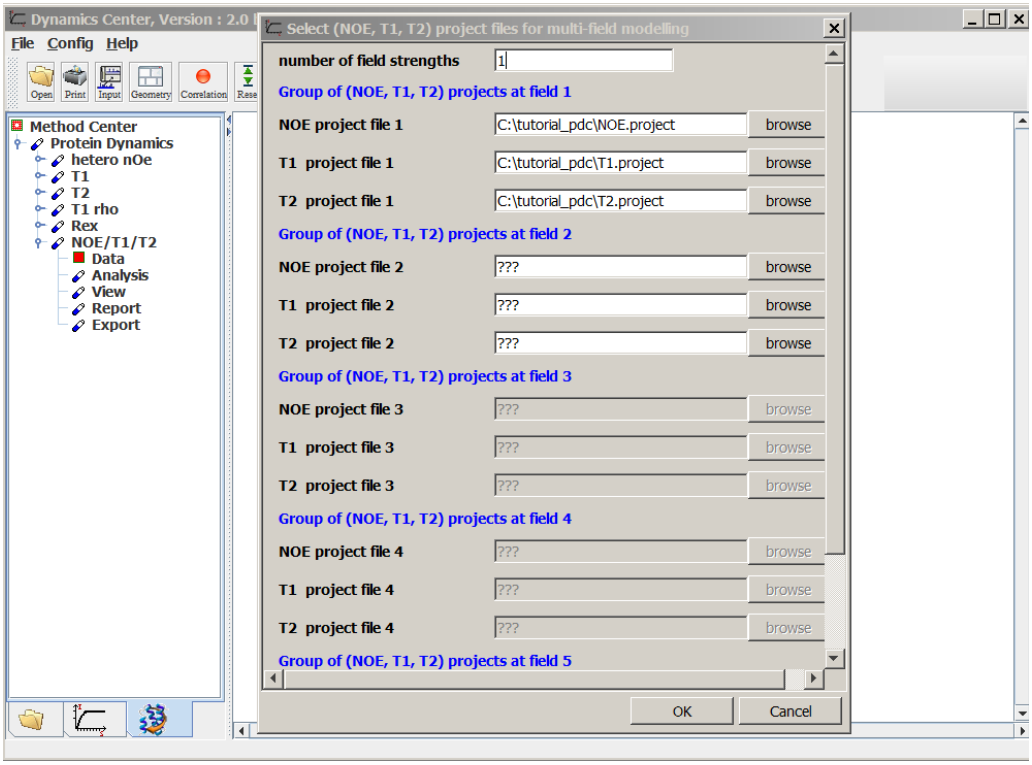
From these basic exercises you learned how to get relaxation parameters. **The procedure is always the same**! Much more is possible, e.g. you can move peaks, manipulate fit curves etc. Perhaps have a look into the hand book available under **Help/Manual**.

Exercise-4: NOE / T1 /T2 Method

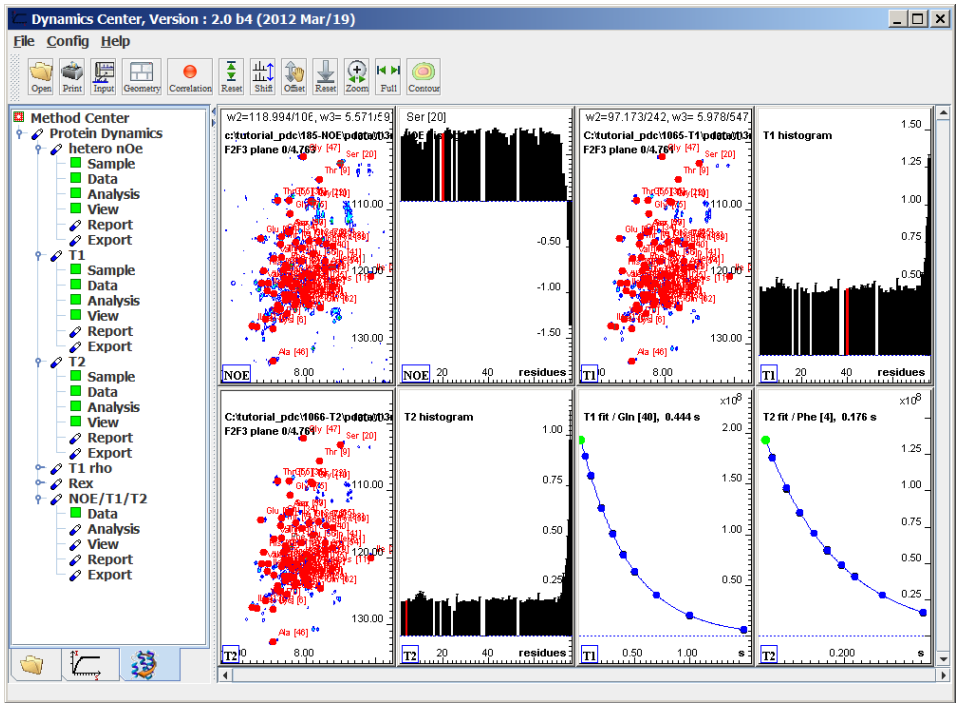
- Repeat the previous exercise, but with T2 (load T1.project into T2, in **Data** update spectrum name to **c:\tutorial_pdc\1066-T2\pdata\1\3rrr**, Analysis, finally save as T2.project) so that 3 project files called **NOE.project, T1.project, T2.project,** are available, e.g. all stored in the folder c:\course_data_pdc

- For simplicity get a blank screen. Use the **Close** option from the popup menus of the opened methods on the method tree to get rid of the various display objects.

- Then open **NOE/T1/T2** with a left mouse button click on the tree, and then **Data**.

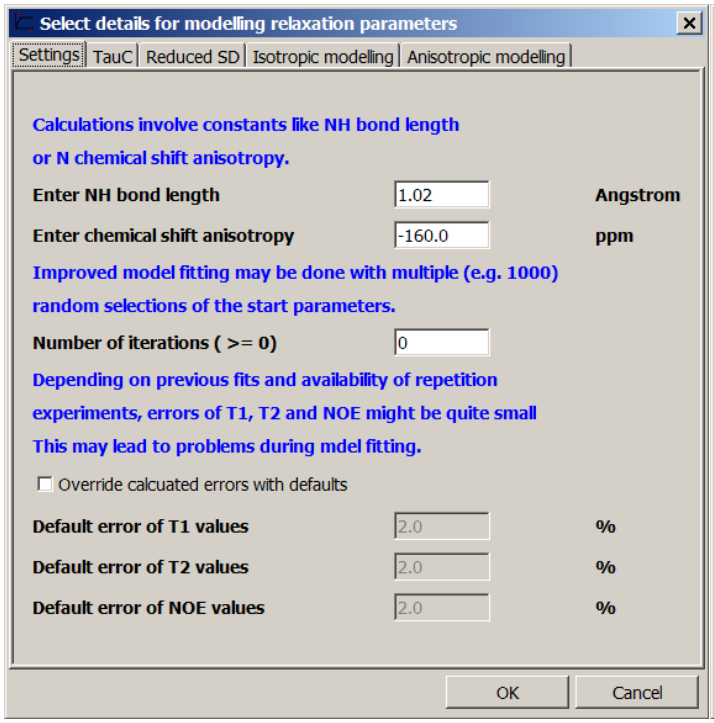


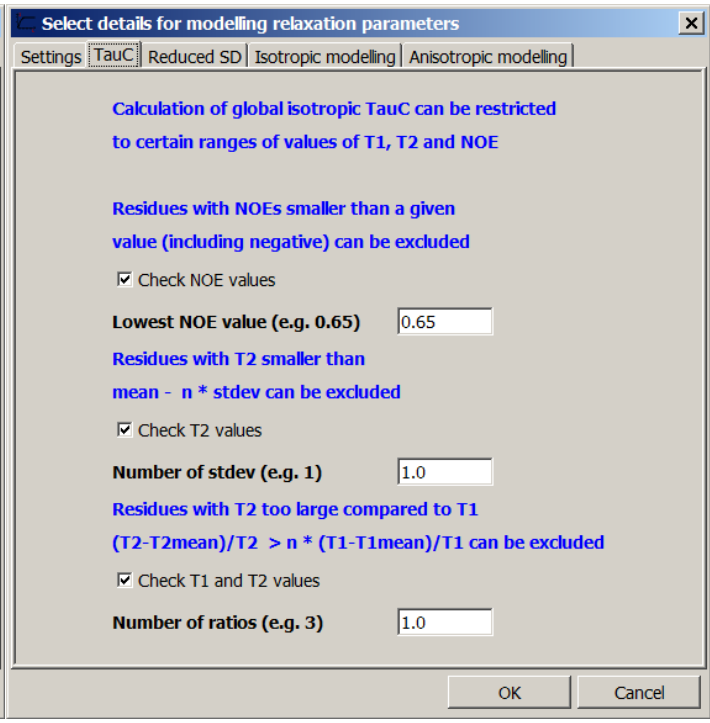
The NOE/T1/T2 method uses already executed NOE, T1 and T2 methods. Therefore it does not need **Sample** and it does not need details on spectra and peaks. Instead just specify the number of field strengths you have available (only 1 here) and the name of the project files to which you had saved the NOE, T1 and T2 projects. The projects are loaded one by one and executed up to **View**. After moving the cursor around in the spectra the resulting display looks like:



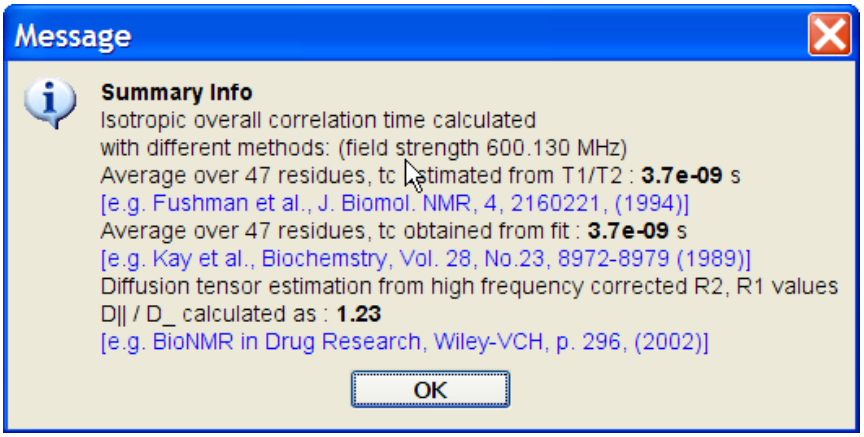
In case it looks different check the **View** options of each method. All objects are active and cursors are linked.

- Now execute **Analysis** of the NOE/T1/T2 method. It is important that sample temperature, amino acid file and structure file are identically set in all methods. This is checked, an error message is displayed if inconsistencies are found. If everything is ok select the details for the modeling:





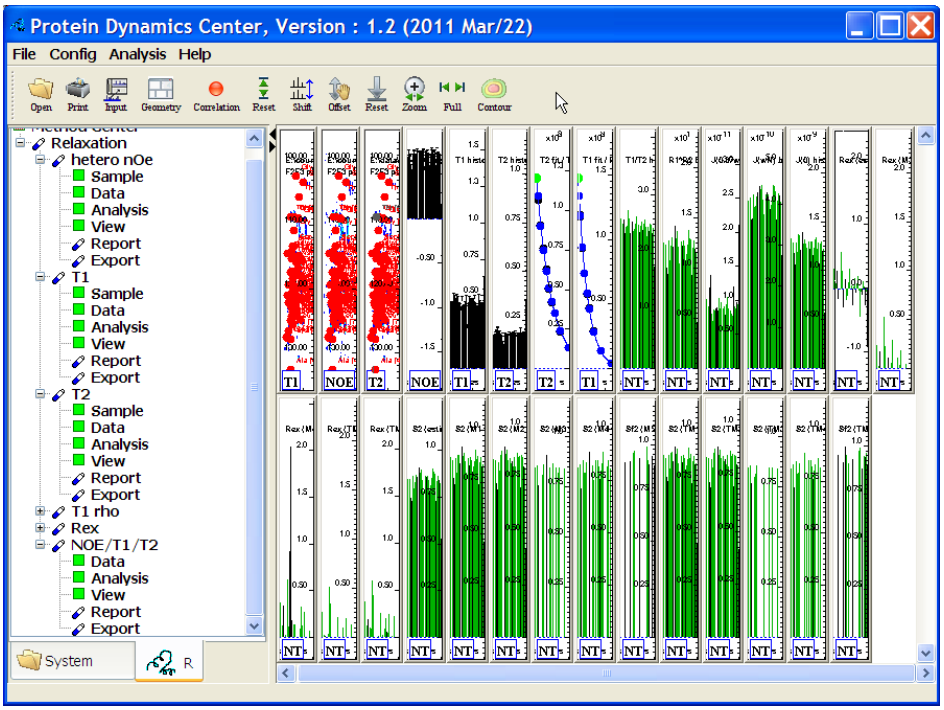
- The first page of the Analysis dialog window contains a few general settings. The second page is used to check NOE, T1 and T2 values to find those residues that do not show fast local motions or undergo conformational exchange. The remaining residues are used to calculate the global correlation time and to estimate the diffusion tensor components. **All other tic boxes on all other pages should be switched on.** After selecting OK the first result display looks like:



The global correlation time as found with different calculations is shown. It will be used for the further modeling. The ratio $\mathbf{D_{\|}/D_{\_}}$ of the diffusion tensor is an estimate. But it is needed to decide whether further isotropic modeling is sufficient (e.g. if < 1.2) or if anisotropic modeling must be done. This requires a PDB file (that includes protons) as specified under **Sample** of the NOE, T1 and T2 methods.

- Since all other tic boxes had been switched on, the modeling will now take a bit of time, perhaps a minute. Under real conditions one would allow the PDC to perform more automated start parameter iterations (see first page of the Analysis dialog window) which then takes even longer.

- Once the modeling calculations are finished use **View** to display results. Activate all tic boxes in the dialog window at first. To simplify the display with the many small windows you can later either switch off individual objects, e.g. don't display the reduced spectral densities or use the **toggle** option from the popup menus on the display to toggle between individual and full displays or use the



visibility option from the popup menus on the display any selection of objects.

- With **Report** and/or **Export** you can get pdf reports, text files or EXCEL .xls files of all details. To get quick information, select **Properties** from popup menu after right click to any histogram.

- To memorize all details use **Save As** from the NOE/T1/T2 popup menu and save to a project file. e.g. NOET1T2.project. The next time you want to execute the method, use **Open** from the popup and specify this name again.

- To simplify the display chose **View** again and switch off unwanted display objects. Alternatively, do a right mouse button click at any object and select **visibility** from the popup many. Individual display objects can be switch on/off at any time. If you accidentally switched off everything, execute **Visibility of objects** from the **File** pull-down menu.

- Via **View** it also possible to switch on a **simple molecular display** and show relaxation or modeling results on the structure in color coded form. Java's **Jmol** is used and opens up a separate window. Jmol understands **Rasmol** commands which can be used to customize the display.