

# CCPNmr Analysis for solid-state MAS NMR Assignment

Please use the project provided, called *sh3\_tutorial*.

## (A) Familiarisation with the Program

### Switching Spectra on/off

- In the top left hand corner of each window there is a **Spectra** button. Toggle this on/off to see all the spectra which can be displayed in each window. Each spectrum can be toggled on/off using its button, so you are able to display as many or as few spectra in each window as you wish.
- Open further windows by going to the **Windows** menu and selecting any of the windows in the lower section of the pull-down menu. Note that you can look at the 3D spectra in two ways: either with the nitrogen dimension along the y axis, or along the z axis. The window names (e.g. NCACX Nz, NCACX CAz) indicate which dimension has been placed along the z axis.
- Note that the spectra are colour coded according to the isotopic labelling of the samples they were recorded on:

black	uniform $^{13}\text{C}$ , $^{15}\text{N}$ labelling
light/dark blue	uniform $^{15}\text{N}$ labelling, 1,3- $^{13}\text{C}$ glycerol labelling
red/pink/purple	uniform $^{15}\text{N}$ labelling, 2- $^{13}\text{C}$ glycerol labelling
- Note the nomenclature of the spectrum names – they indicate spectrum type, mixing time and sample labelling, e.g.

PDSD100_U	PDSD, 100ms mixing time, uniformly labelled sample
PDSD50_13C	PDSD, 50ms mixing time, 1,3- $^{13}\text{C}$ glycerol sample
NCACX200_2C	NCACX, 200ms mixing time, 2- $^{13}\text{C}$ glycerol sample
NCOCX100_13C	NCOCX, 100ms mixing time, 1,3- $^{13}\text{C}$ glycerol sample

### Zooming / Moving around in Spectra

- There are several ways to zoom (note that the mouse-based ones may not work with Windows!):
  - Rotate the mouse wheel
  - Middle-click the mouse while holding down **Shift** and drag the mouse to zoom in/out
  - Use the **Page Up** / **Page Down** keys
- Move to a different part of the spectrum using the scroll bars or simply click on the middle mouse button and drag the spectrum.
- To move through the z-planes of a 3D spectrum, either use the z-plane scroll bar or rotate the mouse wheel while holding down **Ctrl**.
- If you want to go to a specific position on the z-axis of a 3D window, simply type the ppm value into the box in the bottom left hand corner.

## Peak Picking

- The best way to pick peaks is to let the program find the correct peak maximum for you. To do this, simply left-click and drag the mouse over a peak (or several peaks) while holding down **Ctrl** and **Shift**. You can do this both in 2D and 3D spectra. Note that in 3D spectra you may have to move through the z-planes a bit to get close enough to the maximum for the program to be able to find it and pick a peak correctly.
- Sometimes several peaks are overlapped and it is better to place the peaks manually. Simply place the mouse where you want to have your peak and then right-click the mouse and select **Peaks** and then **Add New Peak**.
- A peak is selected when a box is drawn around it. To select a peak either click on it, or drag the mouse over it. To deselect your peaks just left-click the mouse somewhere in a spectrum window. To select several peaks at the same time, drag the mouse over several peaks in one go, or keep the **Shift** button pressed down while you select each peak individually.

## Marks and Rulers

- Often it is useful to draw lines through your spectrum to check whether two peaks occur at the same chemical shift or not.
- A Mark is drawn through all dimensions at the position where it is placed. To draw a Mark, place the mouse where you want it to be and press **m**. If you are close to a peak, then the mark will automatically be drawn through the peak.
- Rulers only go through one dimension. To draw a Ruler, place the mouse where you want it to be and press **v** for a vertical ruler or **h** for a horizontal ruler.
- You can remove all marks and rulers by pressing **n**. Alternatively you can right-click the mouse and go to **Markers** – here you can place marks and rulers or you can selectively remove only Marks or only Rulers.

## (B) Spin System Identification and Assignment

The SH3 domain of chicken  $\alpha$ -spectrin contains three threonines. This section will show you how to identify them based on their chemical shifts, pick their peaks, generate resonances and spin systems for them, and assign their atom and amino acid types. Then you will use the 3D spectra and glycerol labelling pattern to sequence specifically assign the threonines.

For this section you will need to refer to the following Figures:

Figure 1 – *Graph of the characteristic carbon chemical shifts for all 20 amino acids*

Figure 2 – *The glycerol labelling pattern (averages)*

Figure 3 – *The glycerol labelling pattern (individual isotopomers)*

Figure 4 – *Diagrams illustrating the spectrum types used in this tutorial*

Figure 5 – *The three threonine motifs in SH3 and their labelling pattern in the glycerol based samples*

Figure 6 – *Some  $^{13}\text{C}$ - $^{13}\text{C}$  correlation spectra of SH3 and the identity of some peak clusters based on their chemical shifts and the glycerol labelling pattern*

## Identifying and Peak Picking the Threonine Residues

- Based on their characteristic chemical shifts, try to identify the  $C\alpha$ - $C\beta$ ,  $C\alpha$ - $C\gamma 2$ ,  $C\beta$ - $C\gamma 2$ ,  $C\alpha$ -CO and  $C\beta$ -CO cross peaks of the three threonines in the uniform (black) PDS D spectrum and peak pick them (except possibly the  $C\beta$ - $C\gamma 2$  peaks which are not very well separated). Drawing marks through your peaks will help you connect them.

*In the PDS D window, find the three  $C\alpha$ - $C\beta$  cross peaks in the black PDS D spectrum below the diagonal ( $x$ -axis  $\approx 63$  ppm;  $y$ -axis  $\approx 71$  ppm). Use Figure 6a as a guide, if you like. Begin by peak picking the left hand cross peak. Place a mark through this peak (**m**). Look at the threonine  $C\gamma 2$  region at about 20 ppm. There is only one chemical shift (22.1 ppm) at which there are peaks which go through both marks (i.e. the  $C\alpha$ - $C\gamma 2$  and  $C\beta$ - $C\gamma 2$  cross peaks). Peak pick the  $C\alpha$ - $C\gamma 2$  cross peak (22.1 and 65.0 ppm – unfortunately this cross peak has two maxima – just delete one of them). Now look at the PDS D CO window – this shows the carbonyl region of the spectrum. Again there is one chemical shift (175.8 ppm) at which there are peaks which go through both marks. These are the  $C\alpha$ -CO and  $C\beta$ -CO cross peaks. Peak pick both of these. You have now identified one threonine spin system.*

*Now remove your marks (press **n**) and try the same procedure for the other two threonine spin systems.*

- If you are having trouble, then use this chemical shift table to help you find the peaks:

Atom	Thr A	Thr B	Thr C
$C\alpha$	65.0	62.7	61.7
$C\beta$	71.0	70.0	72.0
$C\gamma$	22.2	22.7	21.4
CO	175.8	173.6	174.1

## Assigning Resonances, Spin Systems, Atom Types and Amino Acid Type

- Go to one of the  $C\alpha$ - $C\beta$  cross peaks, place the mouse on it and either press **a** or right-click the mouse and go to **Assign** and then **Assign Spectrum Name**. This will bring up the **Assignment Panel**:

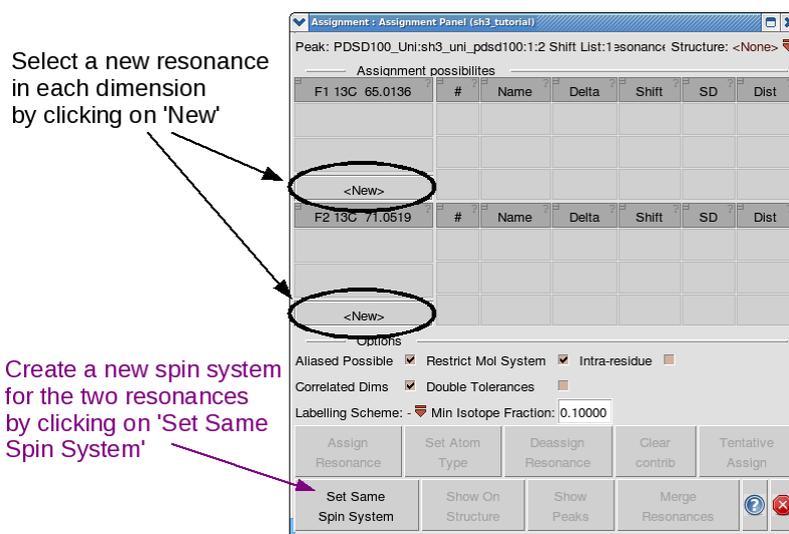
Here the selected assignment for each dimension will be displayed

Assignment options for each dimension will be displayed in this half of the panel

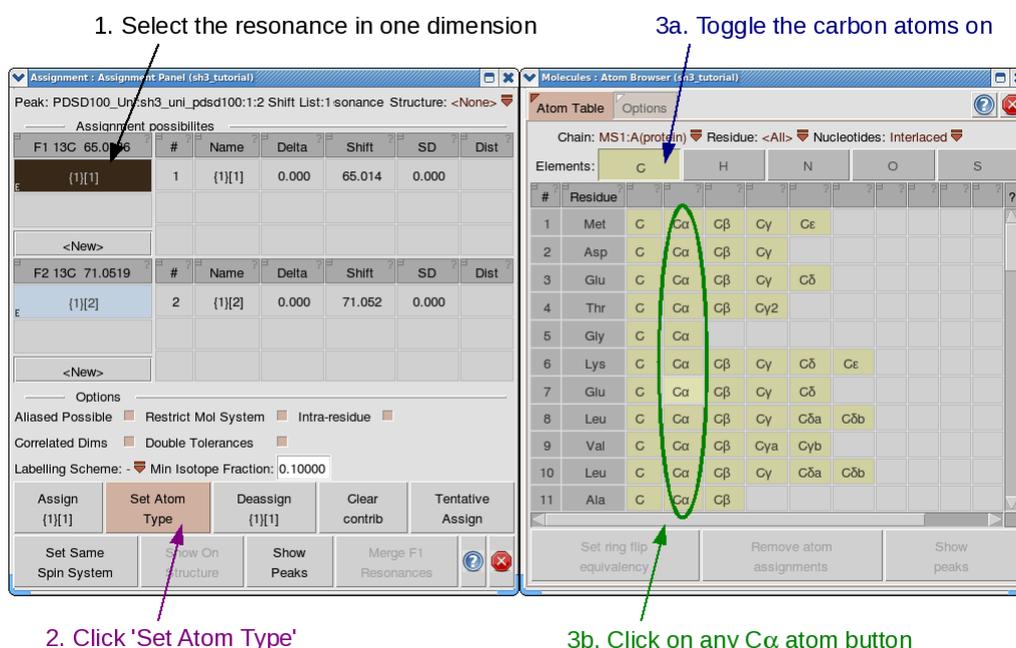
First dimension (x-axis)

Second dimension (y-axis)

- Firstly, select a new resonance for each dimension, by clicking on **New** in both the top and the bottom section of the panel.

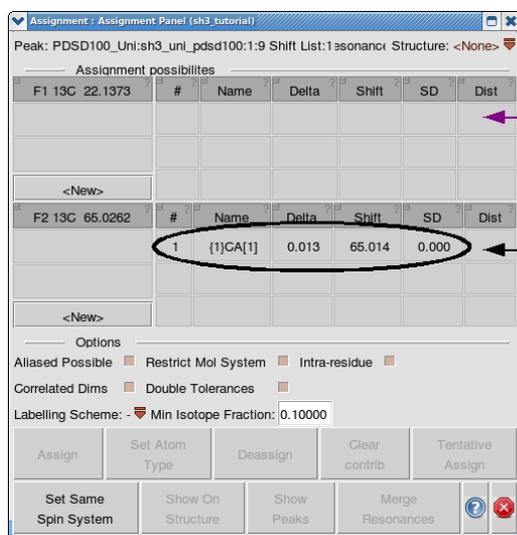


- Secondly, you need to create a new spin system which both of these resonances are part. Simply click on **Set Same Spin System**. Note that the [resonance numbers] are given in square brackets and the {spin system number} is given in curly brackets.
- Thirdly, you need to set the atom type for each of the resonances. Select the top resonance (left hand side) first. Then click on **Set Atom Type** which will bring up the **Atom Browser** panel. Here you will first of all have to toggle on the carbon atoms by clicking on **C**. Then select any **C $\alpha$**  button (it doesn't matter which!). Now do the same for the lower resonance, but selecting **C $\beta$**  as the atom type.



- Repeat this procedure for the other two threonine C $\alpha$ -C $\beta$  cross peaks.
- Now you have three spin systems – one for each of your three threonine residues, and each one contains a C $\alpha$  and C $\beta$  resonance. Now you need to add the C $\gamma$ 2 and CO resonances.

- Find a  $C\alpha$ -CO or  $C\beta$ -CO cross peak, place the mouse on it and press **a**. (Alternatively, right-click the mouse and go to **Assign** and then **Assign Spectrum Name**.) This time the **Assignment Panel** should look like this: for the  $C\alpha/C\beta$  dimension it will give you an assignment possibility. Simply click on this to select it.



2. For this dimension you will have to select a '<New>' resonance, 'Set Same Spin System' and 'Set Atom Type'

1. An assignment option is available in this dimension. Click on this to select it as the assignment

- For the CO dimension you will have to go through the procedure described above to create a new resonance (click **New**), add it to the threonine spin system (click **Set Same Spin System** – make sure this is AFTER you have already assigned the  $C\alpha/C\beta$  dimension, or you will create a new spin system rather than adding your CO to the existing spin system) and assign the atom type (select the resonance, click **Set Atom Type** and then select a **C** atom in the **Atom Browser**).
- Repeat this for all the remaining COs and  $C\gamma$ 2s.
- Because of the unusual threonine chemical shifts, we are sure of our amino acid type. Therefore, we can assign the amino acid type for our three threonine spin systems. Go to the **Resonance** menu and select **Spin Systems**. This pop-up shows you a list of your spin systems. Simply select each of your three threonine spin systems in turn, and click on **Assign Type**. This will bring up the **Atom Browser** and you just need to click on a **Thr** button.

### Identifying the threonine nitrogen chemical shifts

- Now identify the threonine nitrogen chemical shifts using the 2- $^{13}\text{C}$  glycerol NCACX with 100ms mixing time (the NCACX100\_2C spectrum).  
*The easiest way to do this, is to go to the NCACX CAz window and type each threonine  $C\alpha$  chemical shift into the bottom left hand box in turn. Each time you should be able to find a strip which contains a strong N- $C\alpha$ - $C\alpha$  'diagonal' cross peak as well as a slightly weaker N- $C\alpha$ - $C\gamma$ 2 cross peak (use marks in the 2D spectrum to help you find the peaks in the 3D!). If you look at the glycerol labelling scheme you will see that N- $C\alpha$ -CO and N- $C\alpha$ - $C\beta$  cross peaks should not be observable for the threonine in the NCACX spectrum of the 2- $^{13}\text{C}$  glycerol sample, as the  $C\alpha$  and CO or  $C\alpha$  and  $C\beta$  are never simultaneously labelled.*
- Peak pick your N- $C\alpha$ - $C\gamma$ 2 cross peaks and assign the nitrogen chemical shifts using the procedure you used above for the carbon resonances:  
*Bring up the **Assignment Panel** with **a**. Select the  $C\alpha$  and  $C\gamma$ 2 resonances suggested as assignment options. Click on **New** in the nitrogen dimension. Click*

**Set Same Spin System.** Select the nitrogen dimension resonance and click **Set Atom Type** and then select an N atom in the **Atom Browser**.

### Identifying the residues preceding the threonines

- Using the NCOCX500\_13C and NCOCX200\_2C spectra and the graph of standard chemical shifts (Figure 1), can you work out which threonine is preceded by a valine, which by a leucine and which by a serine (see Figure 5)?

*Go through the threonines one at a time. Choose one, and mark all its chemical shifts. Now use the **NCOCX Nz** window and set the z-plane chemical shift to that of your threonine nitrogen chemical shift. Make sure that only the NCOCX500\_13C and NCOCX50\_2C spectra are visible. Use Figure 5 (and Figures 1 and 4) to think about what you would expect to see in an NCOCX of the 1,3-<sup>13</sup>C glycerol sample or the 2-<sup>13</sup>C glycerol sample if the threonine were preceded by a valine, leucine or serine.*

  - *In the nitrogen plane of the threonine which is preceded by a valine, you should only be able to see cross peaks in the NCOCX spectrum of the 1,3-<sup>13</sup>C glycerol sample. The C<sub>γ</sub> resonances of a valine (~ 20 ppm) and the C<sub>α</sub>, C<sub>β</sub>, possibly also C<sub>γ2</sub> resonance of your threonine should be visible.*
  - *For the threonine preceded by the leucine, you should only see peaks in the 2-<sup>13</sup>C glycerol sample: the C<sub>β</sub> and C<sub>γ</sub> resonances of a leucine (~42 and ~27 ppm, respectively) and the C<sub>α</sub>, perhaps also C<sub>β</sub> and C<sub>γ2</sub> of the threonine.*
  - *For the threonine preceded by a serine, cross peaks will again only be visible in the 1,3-<sup>13</sup>C glycerol sample: The C<sub>β</sub> of the serine (~ 64 ppm) and the C<sub>α</sub>, C<sub>β</sub>, possibly also C<sub>γ2</sub> resonance of your threonine.*
- Once you have found the cross peaks in the NCOCX spectra which arise from your threonines, peak pick these.
- You can confirm whether the peaks which you think may arise from valine/leucine/serine residues really do, by marking them and checking whether you can see any corresponding cross peaks in the PDS spectra (see Figure 6 for reference).
  - *For valine you should see a C<sub>γ1</sub>-C<sub>γ2</sub> cross peak (near the diagonal at ~ 20 ppm) in the 1,3-<sup>13</sup>C glycerol spectrum and C<sub>γ1/2</sub>-C<sub>α</sub> (~ 20/62 ppm) and C<sub>γ1/2</sub>-C<sub>β</sub> (~ 20/33 ppm) cross peaks in the uniformly labelled spectrum and a strong C<sub>α</sub>-C<sub>β</sub> cross peak (~ 62/33 ppm) in the 2-<sup>13</sup>C glycerol spectrum.*
  - *For leucine your C<sub>β</sub> and C<sub>γ</sub> should form a strong cross peak in the 2-<sup>13</sup>C glycerol spectrum (~ 42/27 ppm).*
  - *For serine you would expect a C<sub>α</sub>-C<sub>β</sub> cross peak (~ 58/64 ppm) in the uniformly labelled spectrum.*
- In order to assign the cross peaks in the 3D NCOCX spectra, bring up the **Assignment Panel** by holding the mouse over a cross peak and pressing **a**. Your threonine resonances should be provided as assignment options. BUT BEFORE YOU SELECT THESE, generate your new spin systems for the previous valine, leucine or serine:

*Create a new resonances by clicking on **New**. Generate a new valine/leucine/serine spin system or add resonances to a spin system by clicking on **Set Same Spin System**. Assign the atom type by selecting the resonance and clicking on **Set Atom Type** and then choosing the correct atom type in the **Atom Browser**.*

### Further assignment of the EVTMK motif

- Try to identify the methionine which follows this threonine.  
*Use the NCOCX COz window and go to the plane of the threonine CO. In the 2-<sup>13</sup>C glycerol spectrum (200ms) you should be able to see links to the threonine C $\beta$  and methionine C $\alpha$ . To confirm that the C $\alpha$  you have found really is compatible as a methionine C $\alpha$  – see if it matches up with a possible C $\alpha$ -C $\gamma$  cross peak in the 2-<sup>13</sup>C glycerol PDSD spectrum (see Figure 6 for reference). You should be able to find the nitrogen chemical shift of the methionine residue by looking for the N-C $\alpha$ -C $\gamma$  peak in the 2-<sup>13</sup>C glycerol NCACX.*
- At each stage, pick your peaks and assign your resonances and spin systems.
- Now see if you can find links from the methionine to the neighbouring lysine.

### Further assignment of the NSTNK motif

- If you have got to this stage, then you have probably got the hang of things. Try identifying the remaining resonances of the serine spin system and see if you can find links to the preceding asparagine.