

## Series Assign Study Tutorials

This tutorial describes 7 Studies:

- 1) **fastExchange.xipp** Series of 2D  $^{15}\text{N}$ -HSQC on labeled HPr bound to unlabeled EIN where relative concentration of EIN to HPr changes from 0.0 to 1.2 in series.
- 2) **artsy.xipp** ARTSY experiment on GB3 to get  $^1\text{H}$ - $^{15}\text{N}$  residual dipolar couplings.
- 3) **twoPointRate.xipp** Two-point Rate experiment on VPL mutant Fyn SH3.
- 4) **multiPointRate.xipp** Multi-Point Rate on VPL mutant Fyn SH3.
- 5) **pre.xipp** Two-point PRE experiment on HPr bound to unlabeled EIN.
- 6) **multiPointDeltaRate.xipp** Multi-Point Delta-Rate on VPL mutant Fyn SH3.
- 7) **CPMG\_Dispersion.xipp** Experiment Series of 25 2D  $^{15}\text{N}$  HSQC on VPL mutant Fyn SH3 for CPMG Dispersion profile.

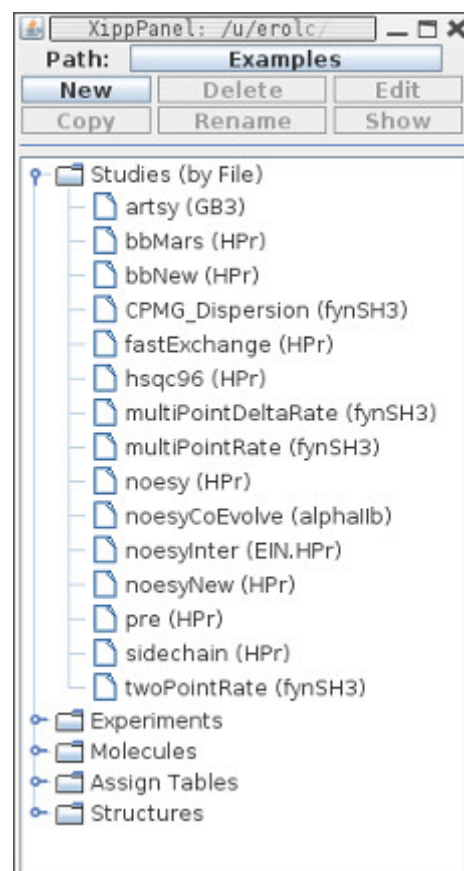
In order to follow along with this tutorial using the example data and study files you need to either install the three files Exmpl\_Linux\_Titration.sh, Exmpl\_Linux\_Artsy.sh and Exmpl\_Linux\_Relaxation.sh or install the single file Exmpl\_Linux\_ALL3D.sh on your Linux computer. The files Exmpl\_Linux\_Titration.sh and Exmpl\_Linux\_Artsy.sh respectively have only the data for the studies fastExchange study and artsy. The file Exmpl\_Linux\_Relaxation.sh has the data for the twoPointRate, multiPointRate, multiPointDeltaRate and CPMG\_Dispersion studies. The file Exmpl\_Linux\_All3D.sh has all of the processed NMR data for all examples excluding the 4D NOEs which are not used in this tutorial.

By default the examples are installed at ~/Xipp/v1/Examples  
Start xipp from the Examples directory so that all of the example studies are immediately available.

On the XippPanel open the 'Studies (by File)' to show all of the Studies defined by the \*.xipp study files in this directory.

To view properties for fastExchange.xipp:  
Select fastExchange (HPr) and click Edit button.

To display NMR data for fastExchange.xipp:  
Select fastExchange (HPr) and click the Show button.  
The Canvas and Table Windows should display quickly since the fastExchange study



## fastExchange.xipp Study Properties

The fastExchange study analyzes  $^{15}\text{N}$  labeled HPr in fast exchange with unlabeled E1N where the ratio of E1N to HPr is 0.0, 0.25, 0.5, 0.75, 1.0 and 1.2 in six samples. This tutorial also demonstrates vector peak-picking to create a set of peak-picks with same peak-ID and label or assignment for the peaks in fast exchange.

Startup File: fastExchange

Study: HPr/Fast-Exchange

Comment:

Peak Assigner Type

Exp. Type: 15N-HSQC-NoNew

Residue/Atom Filters

Active: Properties

Axis Order: HN, N

Axis Name: HN, N

Error Range: HN (0.03), N (0.30)

Assign Offset: HN (0.00), N (0.00)

Enable Residue Jump AutoZoom

Peak Interp...: 3 Point Parabola

Initial View Region: 4

Set Region by:  Calc Union,  Calc Overlap,  Manual

Display-X: HN, Display-Y: N

User Origin: HN (11.37), N (132.945)

User End: HN (4.63), N (100.055)

NMR Data E...: 6

Fra...:  Use Properties,  Same Properties

NMR Data	Show	Off...	Molecu...	Assign...	Structure
Exp 1	<input checked="" type="checkbox"/>	0	HPr	hprAssign	Set Struct...
Exp 2	<input checked="" type="checkbox"/>	0.25	HPr	hprAssign	Set Struct...
Exp 3	<input checked="" type="checkbox"/>	0.5	HPr	hprAssign	Set Struct...
Exp 4	<input checked="" type="checkbox"/>	0.75	HPr	hprAssign	Set Struct...
Exp 5	<input checked="" type="checkbox"/>	1	HPr	hprAssign	Set Struct...
Exp 6	<input checked="" type="checkbox"/>	1	HPr	hprAssign	Set Struct...

OK Cancel

## fastExchange.xipp

This Fast Exchange dialog was one of the earliest dialog panels created to analyze a series of experiments and at some point will be updated to better align with the relaxation series panels.

- 1: Before setting experiment properties define the number of experiments in the fast exchange series.
- 2: Click buttons labeled Exp\_1 through Exp\_6 to individually define each  $^{15}\text{N}$  HSQC experiment in the series. The order in this list will define the order of the Exp buttons in the Canvas window. Although not required you should order the experiments from low to high or high to low with respect to EIN.
- 3: For each experiment enter the expected Fraction Offset (on dialog panel this is truncated to 'Fra... Off...'). This identifies the fraction offset from the first NMR experiment to this experiment. This should be a value between 0.0 and 1.00 to indicate the fractional PPM offset from the PPM position in the first experiment to this experiment. Exp\_1 should always have 0 for fraction offset. The fraction offset value allows vector peak-picking in Xipp to peak-pick an atom whose position changes frequency using a single gesture. The new PeakPick's in all experiments share same peak-pick ID and label, but their PPM and intensity reflect their experiment.
- 4: The 'Initial View Region' defines the full view in the Canvas Window. This can only be set after all of the experiments (1) have been defined. It will get updated after each experiment is set to a default value. Change 'Axis Name' to alter which axis is along the display X and Y. Note must first select 'Unknown' to free up an axis name to use on the other display axis. To ensure that the full SW is viewed click 'Calc Overlap' and then 'Calc Union' to update the 'User Origin' and 'User End' in PPM.
- 5: 'Use Properties' button must be selected in order to make assignments which will use Molecules and Assign Tables. When toggled off none of these properties are used so that when peaks are created the user sets a label for each peak instead of selecting an assignment from a list suggested by Xipp. 'Same Properties' can be enabled so that a single molecule and assignment table is used for all experiments.
- 6: Clicking the button labeled HPr brings up the Molecule Select/Create Dialog that is used to select a previously defined molecule or define a new molecule. Prior to setting the molecule the button is labeled 'Set Molec...'. The name for the molecule must be a unique molecule name in the current study and is used to identify the molecule within Xipp. The names from all molecules in all studies in the current directory are displayed. See Molecule Select/Create Dialog in createSetStudies.pdf for more details. All linked groups have the same molecule name since they have the same sequence.
- 7: Clicking the button labeled hprAssign brings up the Assign Table Select/Create Dialog that is used to select a previously defined assignment table or define a new assignment table. Prior to setting the assignment table the button is labeled 'Set Assig...'. The assignment table should be assignments for fully bound or with no ligand. In this example the assignment table is for HPr with EIN fully bound. See Assign Table Select/Create Dialog in createSetStudies.pdf for more details. All experiments will use the same assignment table, but only Exp\_5 and Exp\_6 will easily find assignments. You might consider creating a separate assignment table for each sample, but this is not needed. Xipp can copy the assignment and peak-ID from one peak to all peaks in the series ensuring that all peaks get assigned.

Clicking OK at the bottom of the Edit Study Dialog will save all of the properties into the file based on the study name, ie fastExchange.xipp in this example.

# fastExchange.xipp

## Edit Exp\_1

1: This will be label on Exp button in Canvas Window. Can keep as is or change to something meaningful such as fraction offset, ie 0, 0.25, etc.

2: Click the NMR Data File button to bring up the NMR Data File Chooser to select the file or file-set. Initially the text on this button will be red for a new study indicating that the file has not been set.

3: 'Axis Order' is determined when NMRPipe file is defined based on SF and gyromagnetic ratio. Note all experiments in series must have same axis order.

4: The 'Assign Offset' defines a PPM value added to the observed peak position to match the chemical shifts in an assignment table. Note all experiments in series must have same assign offset.

5: Clicking the Peak File button brings up the Peak Pick File Chooser Dialog. The initial default value for the peak-pick file name is the base name from the NMR Data file with the extension replaced with .ASG and .PCK.

## fastExchange.xipp Show Study

Refer to the document XippCommands.pdf for general information about keyboard and mouse commands for Xipp.

Ensure that fastExchange (HPr) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the fastExchange Study.

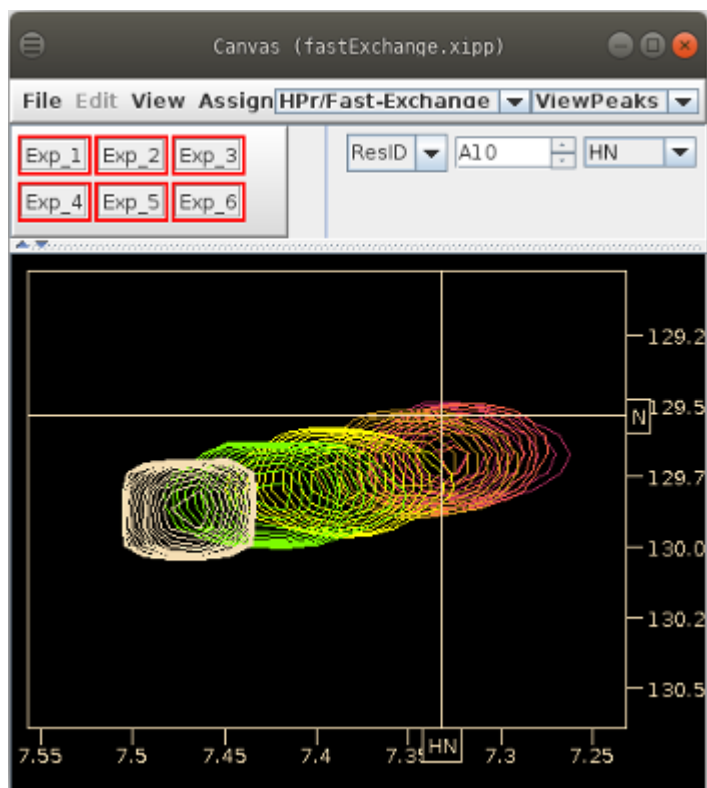
At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Zoom into region 7.4, 129.8 PPM, ie use middle mouse button.
- (2) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (3) Enter Keyboard command: Alt-J 10 to residue jump A10. You can also type 10 into Jump window and hit enter.
- (4) Scroll Shift table down to A10

The Exp buttons on the Canvas window should all be on a single row unless your window size has been drastically reduced which is not recommended unless you are making figures from screen shots and want the text to be legible. The Exp buttons are in two rows only because the window size has been reduced to fit on a page and be able to easily read the text. The display for any experiment is toggled on/off by clicking its Exp button.

The six sets of contours shown all belong to A10 and show the change in chemical shift from the reference experiment Exp\_1 with only  $^{15}\text{N}$  labeled HPr (no EIN) shown in pale white.

The top part of the Table Window is tabbed and shows the ShiftTable. The bottom part always shows information about peaks that have been picked. This is equivalent to the information shown in PIPP's Name mode.



Residue	Atom-Assignment-Table	Shift	RMS
10182-	HA	4.4716	-
10183-	HB	4.3674	-

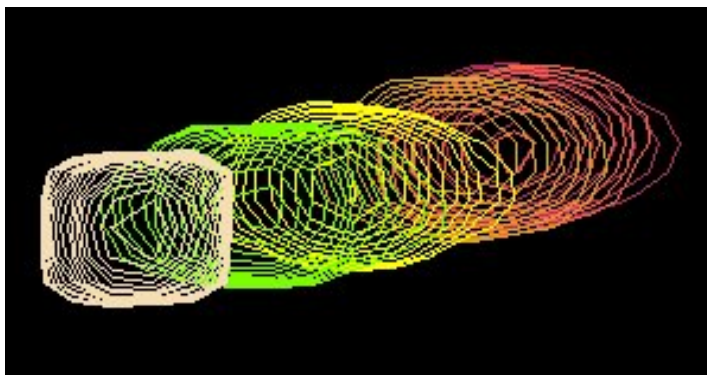
AtomID	AtomName	Shift	RMS
10190-	N	129.5320	-
10191-	HN	7.3323	-
10192-	CA	51.1340	-
10194-	CB	18.7450	-
10195-	HA	4.4819	-

Contours	PkID	Assign	HN	N
	A +-			

Assignment-Table

## fastExchange.xipp Vector Peak Picking



Vector peak picking is done by depressing the left (add peak) or right (delete peak) mouse button starting the mouse at one end of the fast exchange set of peaks and releasing the button after dragging the mouse to the opposite end of the set of peaks. If the Fraction Offset is close to correct for each experiment and the chemical shifts are in fast exchange then Xipp will correctly add a peak in the right place or remove a peak from the right place in each experiment. The distance in PPM units between the peak at the mouse down and mouse up define the magnitude of the shift change. Xipp calculates a starting position in each experiment using the fraction offset and the magnitude of the shift change. The starting position in each experiment is optimized to find nearest largest data point and interpolated via fitting top 3 points to a parabola in the same way as if each experiment was clicked on at the starting location. There is no flag to enable vector peak-picking. If the mouse down and mouse up are within a small number of pixels then regular peak picking is done, otherwise vector peak picking is done. It does not matter which end of the series of peaks is the start since Xipp looks at the absolute magnitude of signal at the two end-points to determine whether the mouse down occurred at Exp\_1 or Exp\_6. The benefit of vector peak picking is that all peaks have the same peak-ID and a single gesture is used to peak-pick all 2D  $^{15}\text{N}$  HSQC experiments. This provides a robust way to peak pick fast exchange spectra.

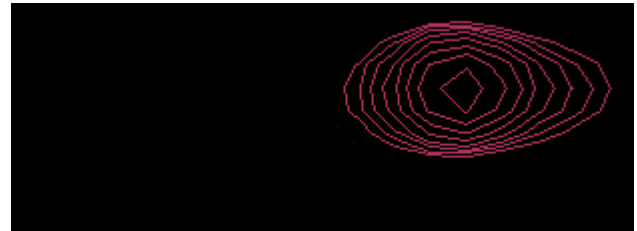
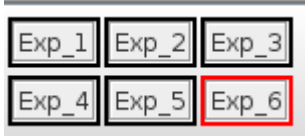
The modifier keys Ctrl and Alt can be used with vector peak picking. The Ctrl key being down has the same meaning in vector peak picking as in regular peak-picking in that no optimization is done on the position of the peak-picks, ie the peaks are placed at exactly the mouse down, mouse up and at precisely the fraction offset from Exp\_1 for experiments 2 through 5. The Alt key is used in regular peak-picking to move a peak-pick. The first step is to copy a peak into the Peak Move Buffer by holding the Alt key down and click left mouse button over a peak-pick. In vector peak-picking if the Alt key is down and there is a peak-pick in the Peak Move Buffer then in addition to having the same peak-ID the assignment or label is copied to all peaks in the fast exchange series. Warning some window managers steal the Alt key with mouse button and Xipp does not see it.

The peak-pick table from a reference experiment can be used label or assign all peaks in the series. The reference experiment does not need match the series since all peaks will be copied/moved. In the first step the Alt key is used to copy a peak into the Peak Move Buffer by holding the Alt key down and click left mouse button over a peak-pick. If the peak-pick to be moved is not close to the top of the peak, ie over noise, the holding the Ctrl (prevent optimization) and the Alt key together when clicking the left mouse button is needed to copy the peak into the Peak Move Buffer. Then only hold the Alt key with left mouse button down at one end of the fast exchange, drag mouse to other end of peaks and release left mouse button and Alt key.

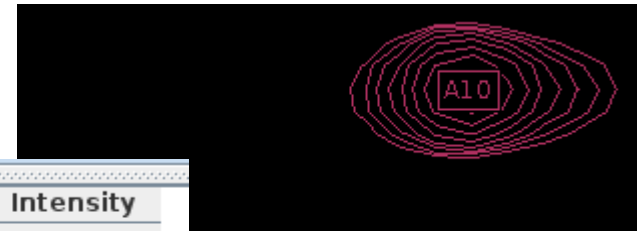
# fastExchange.xipp

## 5 Steps to Copy Assign/Label

1) Click experiment buttons Exp\_1, Exp\_2, Exp\_3, Exp\_4 and Exp\_5 to toggle off all experiments except reference experiment with assignments.



2) Click left mouse button to Peak-pick and assign reference experiment.



Contours	PkID	Assign	HN	N	Intensity
A +- Assignment-Table					
	4	A10.N,HN	7.327	129...	7.392e+5
	K + Exp_6 Assigns (1)		HN	N	
		A10.N,HN	0.0049	-0.1419	

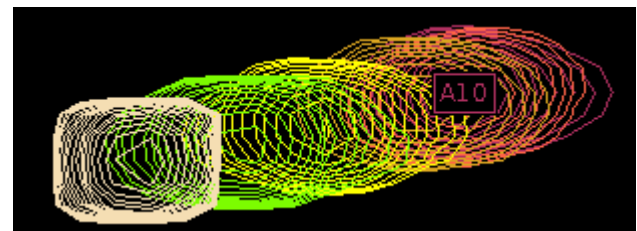
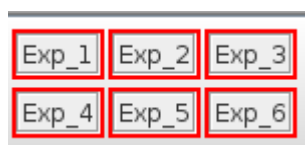
Added to top of table when peak is picked and shows possible assignments.

3) Hold Alt key click left mouse button to copy peak-pick into Peak Move Buffer.

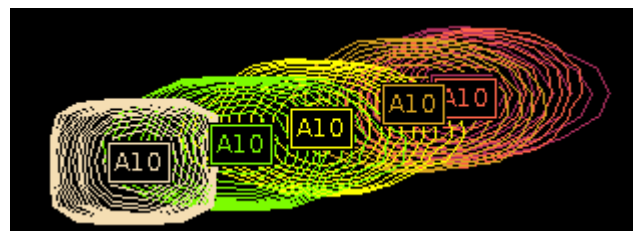
Contours	PkID	Assign	HN	N	Intensity
A +- Assignment-Table					
	4	A10.N,HN	7.327	129...	7.392e+5
	K +				

Added to top of table when peak is copied into Move Peak Buffer and does NOT show possible assignments.

4) Click experiment buttons Exp\_1, Exp\_2, Exp\_3, Exp\_4 and Exp\_5 to toggle on all experiments.



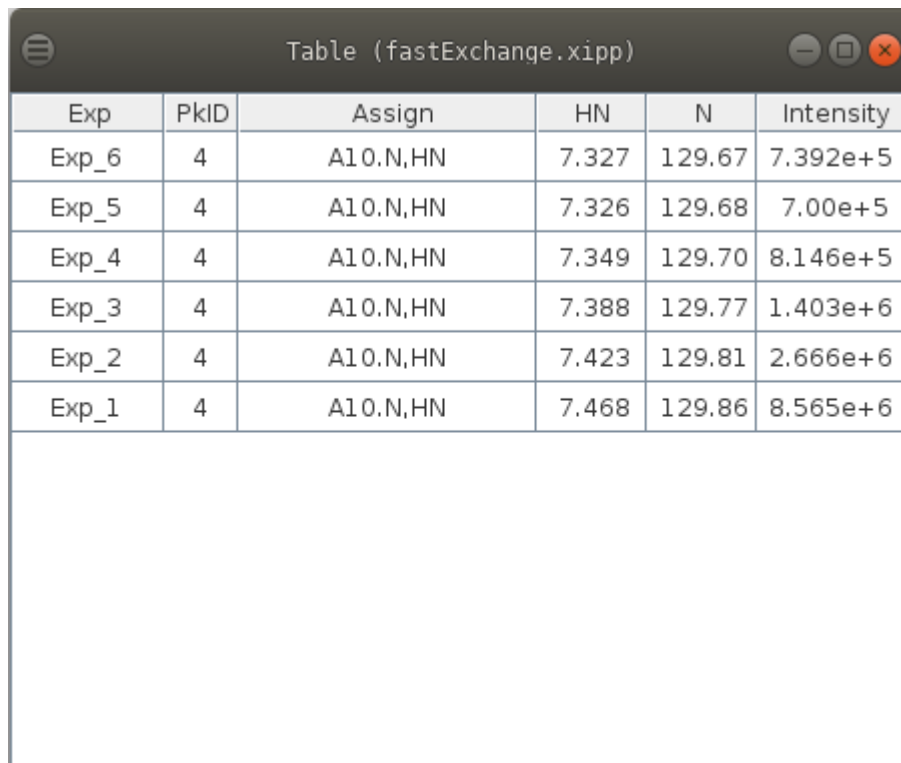
5) Hold Alt key click left mouse down at one end of series, drag mouse to other end of series and release mouse button and Alt key to copy peak-ID and assignment to all experiments in the series.



## fastExchange.xipp

### 5 Steps to Copy Assign/Label

Use the keyboard command 'n' to show the Peak-Pick Table in the Table Window to see that all peaks have the same peak-ID and assignment, but vary HN, N and Intensity.



Exp	PkID	Assign	HN	N	Intensity
Exp_6	4	A10.N,HN	7.327	129.67	7.392e+5
Exp_5	4	A10.N,HN	7.326	129.68	7.00e+5
Exp_4	4	A10.N,HN	7.349	129.70	8.146e+5
Exp_3	4	A10.N,HN	7.388	129.77	1.403e+6
Exp_2	4	A10.N,HN	7.423	129.81	2.666e+6
Exp_1	4	A10.N,HN	7.468	129.86	8.565e+6

It is actually not necessary to toggle off all experiments except the reference experiment in step 1. What is important is that when the reference experiment is peak-picked the nearby experiments are not peak-picked because the assignment does not get copied during vector peak-picking when the peak-pick already exists. How many experiments need to be toggled off depends on the magnitude of shift change, line width and where the mouse is located. It will always work when all experiments except the reference are toggled off, but with experience you should be able to toggle off just 2-3 experiments.



## artsy.xipp Study Properties

The artsy study on GB3 is used to get  $^1\text{H}$ - $^{15}\text{N}$  residual dipolar couplings.

Reference:

N.C. Fitzkee and A. Bax J. Biol NMR (2010) "Facile measurement of  $^1\text{H}$ - $^{15}\text{N}$  residual dipolar couplings in larger perdeuterated proteins." <http://dx.doi.org/10.1007/s10858-010-9441-9>

The screenshot shows the 'Edit Study: artsy' dialog box with the following fields and values:

- Startup File: artsy
- Study: GB3/artsy
- Comment: (empty)
- Series Type: Two-Point (Artsy)
- NMR Data Experiments: Reference (1), Attenuated (2)
- Dephase Time: mS (11) (3)
- Peak Interpolate: 3 Point Parabola -- Same Position (4)
- Enable Residue Jump AutoZoom
- Initial View Region: Set Region by Calc Union (5)
- Axis Name: HN (6), N (7)
- User Origin: 10.999, 135.499
- User End: 5.998, 102.631
- Use Properties (5)
- Same Properties (6)
- Molecule: GB3 (6)
- Assign Table: qb3Assign (7)
- Structure: Set Structure
- Series Model: Two-Point Artsy (8)
- Results in File: Examples/artsyResults.tbl (9)

Buttons: OK, Cancel

## artsy.xipp

- 1: Click button labeled Reference to bring up the Experiment Dialog and set the reference experiment properties. After clicking OK on reference experiment dialog the Initial view region should get updated.
- 2: Click button labeled Attenuated to bring up its Experiment Dialog to set its experiment properties.
- 3: The dephasing time in mS.
- 4: The peak-pick interpolation is pre-set to '3 Point Parabola – Same Position' for Artsy. This fit the top 3 points in the Reference experiment to a parabola and use the same position with no optimization to peak-pick the Attenuated experiment. The Attenuated uses 'Same Position' because its signal will be weak and in some cases under the contour threshold.
- 5: 'Use Properties' button must be selected in order to make assignments which will use Molecules and Assign Tables. When toggled off none of these properties are used so that when peaks are created the user sets a arbitrary label for each peak instead of selecting an assignment from a list suggested by Xipp. 'Same Properties' is enabled by default so that a single molecule and assignment table is used for all experiments.
- 6: Clicking the button labeled GB3 brings up the Molecule Select/Create Dialog that is used to select a previously defined molecule or define a new molecule. Prior to setting the molecule the button is labeled 'Set Molec...'. The name for the molecule must be a unique molecule name in the current study and is used to identify the molecule within Xipp. The names from all molecules in all studies in the current directory are displayed. See Molecule Select/Create Dialog in createSetStudies.pdf for more details. All linked groups have the same molecule name since they have the same sequence.
- 7: Clicking the button labeled gb3Assign brings up the Assign Table Select/Create Dialog that is used to select a previously defined assignment table or define a new assignment table. Prior to setting the assign table the button is labeled 'Set Assig...'. See Assign Table Select/Create Dialog in createSetStudies.pdf for more details.
- 8: Clicking button labeled Two-Point Artsy brings up the Artsy Series Model dialog described below.
- 9: The Artsy results file can be changed only in the Artsy Series Model dialog. The file name I shown here for convenience.

Clicking OK at the bottom of the Edit Study Dialog will save all of the properties into the file based on the study name, ie artsy.xipp in this example.

## artsy.xipp Edit Experiment: Reference

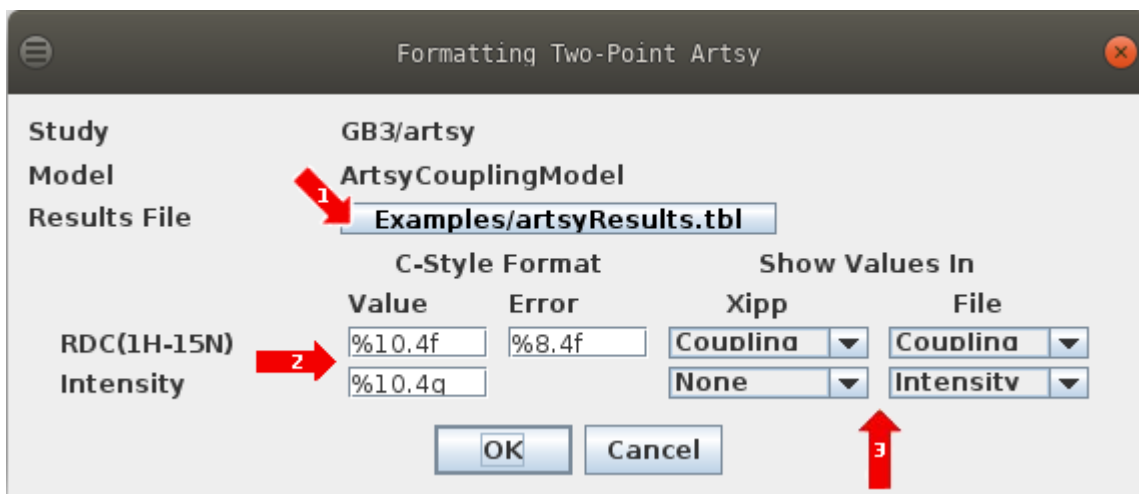
The dialog box contains the following fields and controls:

- Study:** GB3/artsy
- Comment:** (empty)
- Exp. Name:** Reference
- NMR Data File:** test001.ft2 (button), Reference (button), Error Estimate: 0 (input)
- Exp. Type:** 15N-HSQC-NoNew (button)
- Residue/Atom Filters:**  Active, Properties (button)
- Axis Order:** HN (dropdown), N (dropdown)
- Axis Name:** HN (0.03), N (0.30) (Error Range); HN (0.00), N (0.00) (Assign Offset)
- Peak Interpolate:** 3 Point Parabola (button)
- Peak File:** reference reTest.ASG (button), Auto-Saver (button)
- Contour Level:** Level: 5.000e+06 (input), Multiplier: 1.3 (input), Calculate:  All,  Positive,  Negative
- Use Extrema:**  Level: 8.0e+05 (input), Path: /u/erolc/Xipp/ExampleData/GB3/Artsy (input), File Name: test001.XTRMA (input)
- Buttons:** OK, Cancel

- 1: Click the NMR Data File button to bring up the NMR Data File Chooser to select the file or file-set. Initially the text on this button will be red for a new study indicating that the file has not been set.
- 2: 'Axis Order' is determined when NMRPipe file is defined based on SF and gyromagnetic ratio.
- 3: Click the Peak File button to bring up the Peak Pick File Chooser Dialog. Note the Peak Interpolate button is grayed out because interpolation is defined for the series not an individual experiment.
- 4: Set contour threshold level and multiplier as needed.

Click OK at the bottom of the Dialog to save all of the experiment properties.

## artsy.xipp Two-Point Artsy Series Model



- 1: Click the NMR Results File button to bring up the File Chooser to select the results file.
- 2: Enter new C-Style format if different format is needed.
- 3: The 'Show Values In' buttons for the Artsy study currently have only one option except for Intensity in File which can be Intensity or None.

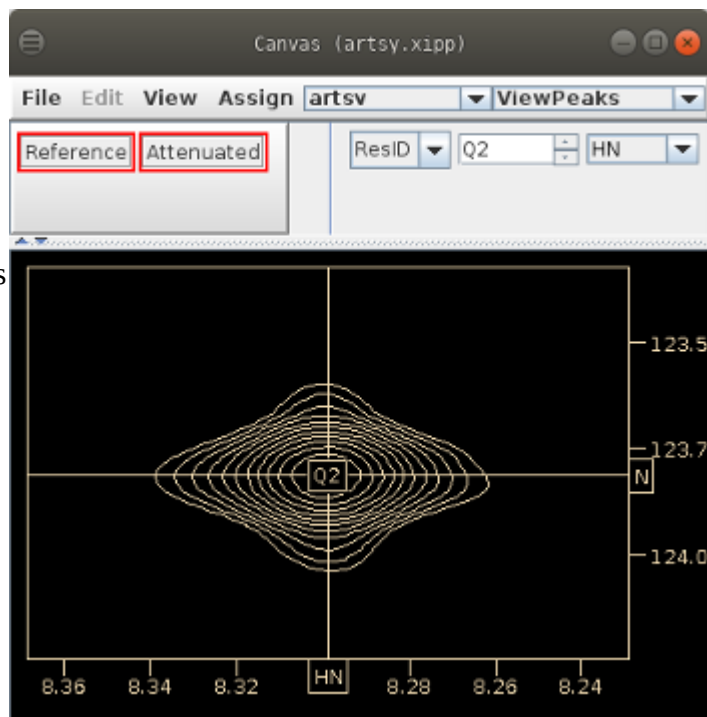
Click OK at the bottom of the Dialog to save the series model properties.

## artsy.xipp Show Study

Ensure that artsy (GB3) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the artsy Study.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (2) Enter Keyboard command: Alt-J 2 to residue jump Q2. You can also type 2 into Jump window and hit enter.
- (3) Click left mouse button on peak at 8.30, 123.8 PPM to Peak-Pick and assign Q2



The contours shown in pale white are from the Reference experiment. No contours for Q2 are observed from the Attenuated experiment since the signal is below the contour threshold.

The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The bottom part always shows information about peaks that have been picked. As peak-picks are created they are added to the top of the Name-Peak Table. The Reference peak-pick was added first and is shown at the bottom with the peak-pick centered on the contours. The Attenuated peak-pick was added second and is at the top of the Name-Peak Table with no contours shown since the threshold for contouring is higher than the peak. A Peak-Pick is created for the Attenuated experiment because its Interpolation is set to 'Same Position' which will always create a Peak-Pick at the same position when the Reference experiment creates a Peak-Pick.

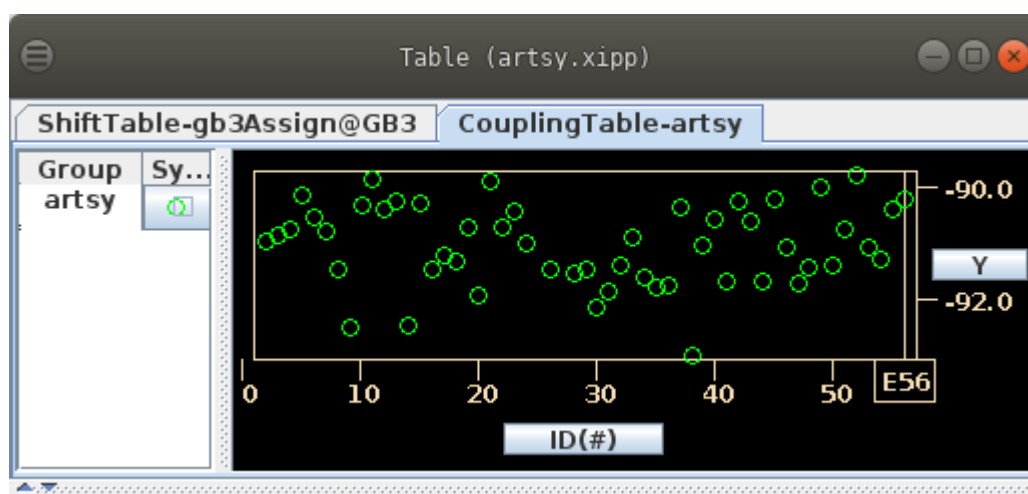
Residue	AtomID	AtomName	Shift	RMS	Count
Q2+	10043-	N	123.8110	-	-
	10044-	HN	8.2990	-	-
Y3+	10059-	N	124.7240	-	-
	10060-	HN	8.9920	-	-

Contours	PkID	Assign	HN	N	Intensity
+	1	Q2.N,HN	8.300	123.812	-1.103e+5
	K+	Attenuated Assigns (...)		HN	N
+	1	Q2.N,HN	8.300	123.812	9.604e+7
	K+	Reference Assigns (1)		HN	N

## artsy.xipp Coupling Table Plot/File

CAPP can not be used with ARTSY data since it will peak-pick only one experiment at a time (Reference or Attenuated) and will not use the 'Same-Position' peak interpolation as is done via manual peak-picking. Manual peak-picking must be done by pointing the mouse at each peak and clicking the left mouse button. However, NH<sub>2</sub>'s or unassigned amino acids will show a peak ID and not get assigned. The Alt key can be held down while clicking the up-arrow key to scroll to the next residue, ie from Q2 to Y3, which will place horizontal and vertical lines at <sup>15</sup>N and HN chemical shift from the ShiftTable for the residue scrolled to and zoom in to the region around the peak. After manually peak-picking and assigning residues Y3 to E56 by using Alt up arrow followed by clicking left mouse button the CouplingTable-artsy tab of the Table Window show a plot of all of the coupling constants.



The results file artsyResults.tbl is automatically created and updated while the experiments are peak-picked. Here is a portion of the results file showing the tabulated data:

```
VARs  KEY RATE ERROR INTENSITY_1 INTENSITY_2  
FORMAT %4d %10.4f %8.4f %10.4g %10.4g
```

```
# Key Coupling Error Ref. Atten.  
2 -90.9423 0.0000 9.604e+07 -1.103e+05  
3 -90.8465 0.0000 1.983e+08 4.289e+05  
4 -90.7247 0.0000 1.905e+08 1.214e+06  
5 -90.1120 0.0000 1.750e+08 4.819e+06
```

The column Key is the residue number.

## twoPointRate.xipp Two-Point Rate Study

Startup File: twoPointRate

Study: fynSH3/rate

Comment:

Series Type: Two-Point T1

NMR Data Exps: Test

At Time(t1): Exp t1

At Time(t2): Exp t2

delta T - t2 - t1: mS 20

Peak Interpolate: 3 Point Parabola

Enable Residue Jump AutoZoom

Initial View Region

Set Region by:  Calc Union  Calc Overlap  Manual

Display-X: HN Display-Y: N

User Origin: 10.201 132.163

User End: 6.497

Use Properties

Molecule: fynSH3

Assign Table: fynSH3wt

Structure: Set Structure

Series Model: Two-Point Rate

Results in File: Examples/twoPointRateResults.tbl

OK Cancel

1: The Series Type is fixed to Two-Point and the Sub-Type can be set to T1, T2 and T1p. Changing the Sub-Type has no effect on anything at this time.

2: Click buttons Exp\_t1 and Exp\_t2 to bring up the Edit Experiment Dialog to define the properties of the 2D <sup>15</sup>N HSQC NMR experiments at the two time points t1 and t2.

3: Enter the delta time (t2 – t1) that relaxation was allowed to occur. The units can be set to mS or S.

4: 'Use Properties' button must be selected in order to make assignments. The button fynSH3 allows defining the sequence and the button fynSH3wt allows defining the assignment table.

5: Click the Two-Point Rate button to bring up the Two-Point Rate Series Model dialog which allows setting formatting and selecting results file.

Clicking OK at the bottom of the Edit Study Dialog will save all of the properties into the file based on the study name, ie twoPointRate.xipp in this example.

## twoPointRate.xipp

### Edit Experiment: Exp\_t1

The screenshot shows the 'Edit Experiment: Exp\_t1' dialog box with the following configuration:

- Study:** fynSH3/rate
- Comment:** (empty)
- Exp. Name:** Exp t1
- NMR Data File:** test001.ft2 (Reference) Error Estimate: 0
- Exp. Type:** 15N-HSQC-NoNew
- Residue/Atom Filters:**  Active Properties
- Axis Order:** HN (dropdown) N (dropdown) ← 2
- Axis Name:** HN, N
- Error Range:** HN: 0.03, N: 0.30
- Assign Offset:** HN: 0.00, N: 0.00
- Peak Interpolate:** 3 Point Parabola ← 3
- Peak File:** test2PR001.ASG (Auto-Saver)
- Contour Level:** Level: 8.000e+05 ← 4, Multiplier: 1.3
- Calculate:**  All  Positive  Negative
- Use Extrema:**
- Level:** 8.0e+05
- Path:** ExampleData/GroEL/20140414 GroEL/53/ft
- File Name:** test001.XTRMA

Buttons: OK, Cancel

1: Click the NMR Data File button to bring up the NMR Data File Chooser to select the file or file-set. Initially the text on this button will be red for a new study indicating that the file has not been set.

2: 'Axis Order' is determined when NMRPipe file is defined based on SF and gyromagnetic ratio.

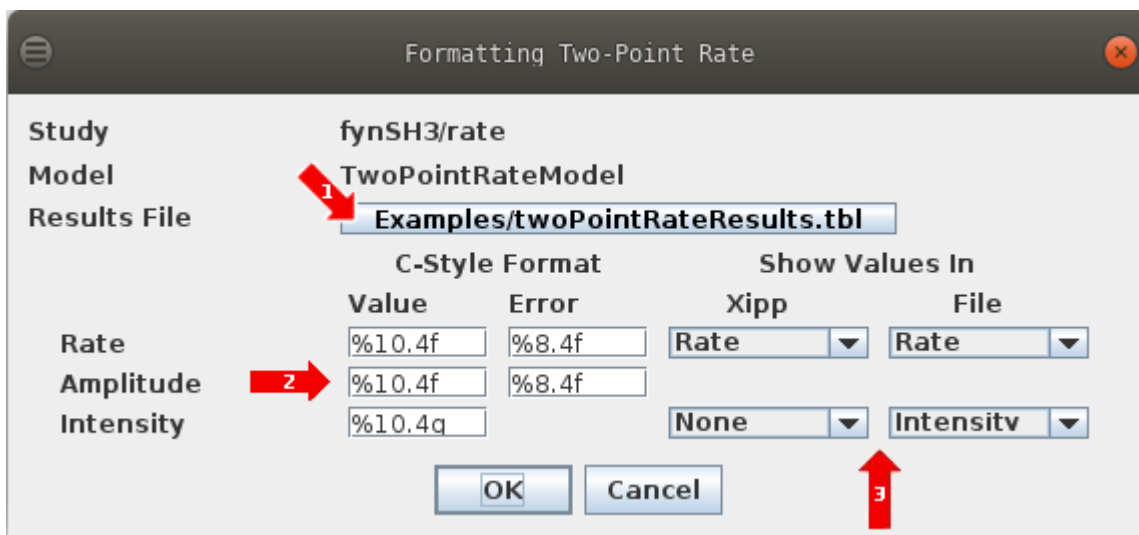
3: Click the Peak File button to bring up the Peak Pick File Chooser Dialog. Note the Peak Interpolate button is grayed out because interpolation is defined for the series not an individual experiment.

4: Set contour threshold level and multiplier as needed.

Click OK at the bottom of the Dialog to save all of the experiment properties.



## twoPointRate.xipp Two-Point Rate Series Model



The screenshot shows a dialog box titled "Formatting Two-Point Rate". The fields are as follows:

Property	Value
Study	fynSH3/rate
Model	TwoPointRateModel
Results File	Examples/twoPointRateResults.tbl
C-Style Format	
Value	%10.4f
Error	%8.4f
Show Values In	
Xipp	Rate
File	Rate
Rate	%10.4f
Amplitude	%10.4f
Error	%8.4f
Intensity	%10.4g
Xipp	None
File	Intensity

Buttons: OK, Cancel

- 1: Click the NMR Results File button to bring up the File Chooser to select the results file.
- 2: Enter new C-Style format if different format as needed.
- 3: The 'Show Values In' buttons for the Two-Point Rate study currently have only one option except for Intensity in File which can be Intensity or None.

Click OK at the bottom of the Dialog to save the two-point model series properties.

## twoPointRate.xipp Show Study

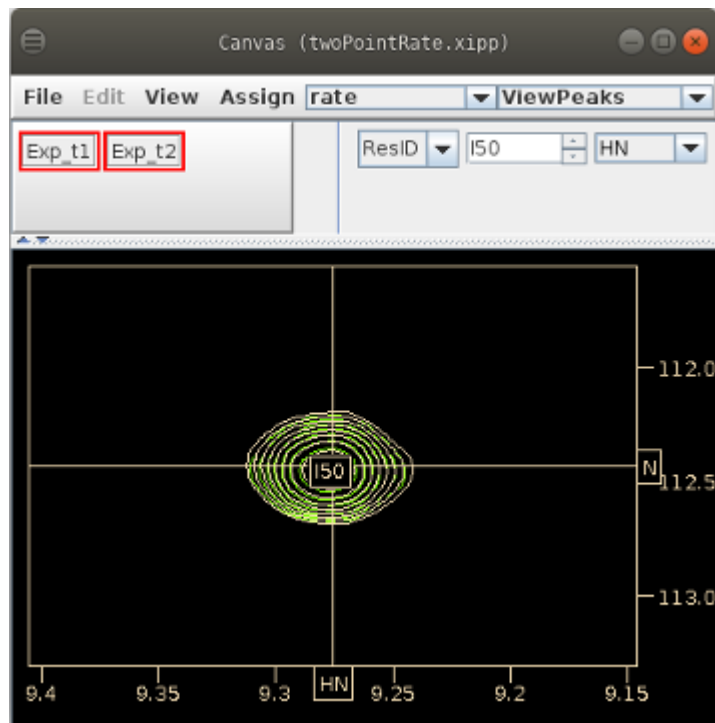
Ensure that twoPointRate (fynSH3) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the Two-Point Rate Study.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (2) Enter Keyboard command: Alt-J 50 to residue jump I50. You can also type 50 into Jump window and hit enter.
- (3) Click left mouse button on peak at 9.3, 112.5 PPM to Peak-Pick and assign I50.

The Exp\_t1 contours shown in pale white are displayed on top of Exp\_t2 contours shown in green.

The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The bottom part always shows information about peaks that have been picked. As peak-picks are created they are added to the top of the Name-Peak Table. The Exp\_t1 peak-pick was added first and is shown at the bottom with the peak-pick centered on the pale white contours. The Exp\_t2 peak-pick was added second and is at the top of the Name-Peak Table with green contours.



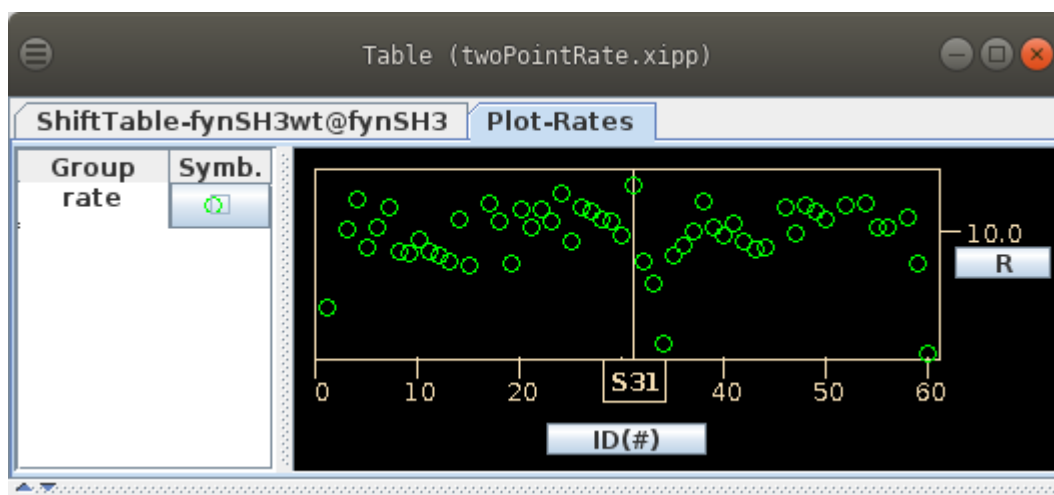
Residue	Atom-Assignment-Table	AtomID	AtomName	Shift	RMS	Count
Y49+	N	10832-	N	118.8930	-	-
	HN	10833-	HN	8.7050	-	-
I50+	N	10852-	N	112.4320	-	-
	HN	10853-	HN	9.2760	-	-
S52+	N	10887-	N	122.0600	-	-
	HN	10888-	HN	7.6930	-	-

Contours	PkID	Assign	HN	N	Intensity
Assignment-Table					
	1	I50.N,HN	9.277	112.451	5.195e+6
	K+	Exp_t2 Assigns (1)	HN	N	
		I50.N,HN	-0.0013	-0.0193	
	1	I50.N,HN	9.277	112.450	6.414e+6
	K+	Exp_t1 Assigns (1)	HN	N	
		I50.N,HN	-0.0013	-0.0185	

## twoPointRate.xipp Rate Table Plot/File

CAPP should not be used with any Series data since it will peak-pick only one experiment at a time and will not ensure the same peak ID between experiments. Manual peak-picking should be done by pointing the mouse at each peak and clicking the left mouse button. However, NH<sub>2</sub>'s or unassigned amino acids will show a peak ID and not get assigned. The Alt key can be held down while clicking the up-arrow key to scroll to the next residue, ie from S1 to R60, which will place horizontal and vertical lines at <sup>15</sup>N and HN chemical shift from the ShiftTable for each residue scrolled to and zoom in to the region around the peak. For each residue a single mouse click peak-picks Exp\_t1, Exp\_t2, assigns each peak-pick, updates the plot and results file. The Plot-Rates tab of the Table Window after manually peak-picking and assigning residues T2 to R60 is shown below.



The results file twoPointRateResults.tbl is automatically created and updated while the experiments are peak-picked. Here is a portion of the results file showing the tabulated data:

```

VARS  KEY RATE ERROR INTENSITY_1 INTENSITY_2
FORMAT %4d %10.4f %8.4f %10.4g %10.4g

```

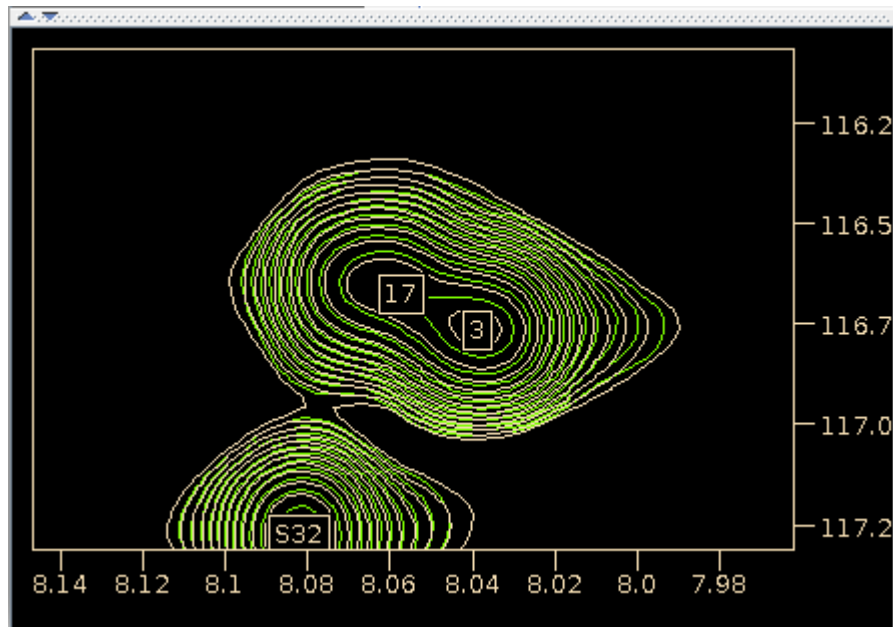
```

# Key   Rate   Error   I(1)   I(2)
1    7.0435  0.0000  1.324e+07  1.150e+07
3   10.1109  0.0000  1.099e+07  8.974e+06
4   11.2945  0.0000  7.350e+06  5.864e+06
5    9.4356  0.0000  7.699e+06  6.375e+06
6   10.2178  0.0000  9.837e+06  8.019e+06
7   10.9956  0.0000  6.070e+06  4.872e+06
8    9.2299  0.0000  1.042e+07  8.663e+06
9    9.1764  0.0000  1.123e+07  9.350e+06
10   9.7144  0.0000  9.234e+06  7.604e+06
11   9.2438  0.0000  9.709e+06  8.070e+06
12   9.1293  0.0000  1.106e+07  9.212e+06
13   8.8329  0.0000  5.596e+06  4.690e+06

```

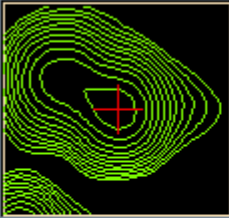
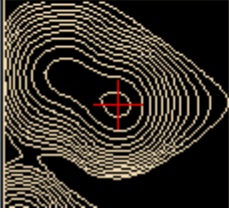
The column labeled Key is the residue number.

## twoPointRate.xipp Assigning Ambiguous



The  $^{15}\text{N}$  HSQC spectrum of the VPL mutant Fyn SH3 is very well resolved with all peaks automatically assigned except for peak IDs 3 & 17. Click the left mouse button on the Canvas Window over peak ID 3 to select peak ID 3 which should insert it from Exp\_t1 and Exp\_t2 into the top of the Name-Peak Table.

On the Table Window in the Name-Peak Table shown below assign peak ID 3 in Exp\_t2 and the corresponding peak ID 3 in Exp\_t1 to T2 by selecting the assignment T2.N,HN for both experiments.

Contours	PkID	Assign	HN	N	Intensity
	<b>3</b>	<b>T2.N,HN</b>	<b>8.039</b>	<b>116.758</b>	<b>1.319e+7</b>
	<b>K+</b>	Exp_t2 Assigns (2)	HN	N	
		T2.N,HN	-0.0018	-0.0142	
		D16.N,HN	0.0202	-0.1212	
	<b>3</b>	<b>T2.N,HN</b>	<b>8.039</b>	<b>116.759</b>	<b>1.549e+7</b>
	<b>K+</b>	Exp_t1 Assigns (2)	HN	N	
		T2.N,HN	-0.0019	-0.0153	
		D16.N,HN	0.0201	-0.1223	

Do the same for peak ID 17. Since peak ID 3 is more intense the mouse should be placed above and to the left of peak ID 17 before clicking the left mouse button otherwise when Xipp looks for a local extrema it will always find peak ID 3.

# multiPointRate.xipp

## Multi-Point Rate Study

The screenshot shows the 'Edit Study: multiPointRate' dialog box. The title bar reads 'Edit Study: multiPointRate'. The 'Startup File' is 'multiPointRate'. The 'Study' is 'fynSH3/rate'. The 'Comment' field is empty. The 'NMR Data Series' is 'test%03d.ft2', with 'Start' at 1 and 'End' at 6. The 'Default Exp. Type' is '15N-HSQC-NoNew'. The 'Series Type' is 'Time', 'T1', and 'Value Units' is 'mS'. A checkbox 'Extract value from NMRPipe' is checked, with a value of 199. The 'dt = t2 - t1' is 'mS' and '33'. The 'Peak File Path' is '53/ft', 'Peak File Format' is 'test%03d MPR.A', and 'Peak Interpolation' is '3 Point Parabola'. The 'Contour Threshold' 'Level' is '8.000e+05' and 'Multiplier' is '1.3'. The 'Calculate' options are 'All', 'Positive', and 'Negative', with 'All' selected. The 'Enable Residue Jump AutoZoom' checkbox is checked. The 'Initial View Region' 'Set Region by' is 'Calc Union'. The 'Axis Name' are 'HN' and 'N'. The 'User Origin' are '10.201' and '132.163'. The 'User End' are '6.497' and '107.249'. The 'Use Properties' checkbox is checked. The 'Molecule' is 'fynSH3', 'Assign Table' is 'fynSH3 WT', and 'Structure' is 'Set Structure'. The 'Update All Exps' and 'EditAllExps' buttons are visible. The 'Series Model' is 'Multi-Point Rate' and 'Results in File' is 'Examples/multiPointRateResults.tbl'. The 'OK' and 'Cancel' buttons are at the bottom.

Startup File: multiPointRate

Study: fynSH3/rate

Comment:

NMR Data Series: test%03d.ft2 Start: 1 End: 6

Default Exp. Type: 15N-HSQC-NoNew

Series Type: Time T1 Value Units: mS

Extract value from NMRPipe: 199

dt = t2 - t1: mS 33

Peak File Path: 53/ft Peak File Format: test%03d MPR.A

Peak Interpolation: 3 Point Parabola

Contour Threshold

Level: 8.000e+05

Multiplier: 1.3

Calculate:  All  Positive  Negative

Enable Residue Jump AutoZoom

Initial View Region

Set Region by:  Calc Union  Calc Overlap  Manual

Axis Name	Display-X	Display-Y
HN	10.201	132.163
N	6.497	107.249

Use Properties

Molecule: fynSH3

Assign Table: fynSH3 WT

Structure: Set Structure

Update All Exps EditAllExps

Series Model: Multi-Point Rate

Results in File: Examples/multiPointRateResults.tbl

OK Cancel

The same Java class, ExpSeriesStudyPanel.java, is used for the Multi-Point Rate, Multi-Point PRE, DEST and CPMG Study Dialog Panel. The delta time (dt) option is not used by Multi-Point Study.

## **multiPointRate.xipp**

### **Multi-Point Rate Study**

- 1: Click the button test%03d.ft2 to bring up the Series NMR Data File-Set Chooser Dialog to define the series of the 2D <sup>15</sup>N HSQC NMR files of the series. The file-set name must include C-style formatting starting with % to position file in series. Start and End numbers are updated after selecting a series.
- 2: The Series Type can be Series, Time or Tau. Must use Time for Multi-Point Rate. The Sub-Type can be set to T1, T2 or T1p. Changing the Sub-Type has no effect on any calculation yet. The Value Units should match the units set in NMRPipe header if Extract value from NMRPipe is enabled. Changing Series Type/Sub Type will erase manually entered relaxation delay in Edit All Exps. Do NOT change Series Type/Sub-Type after manually entering relaxation delay in Edit All Exps Dialog.
- 3: Extract value from NMRPipe indicates that the relaxation delay has been set in the NMRPipe header at position 199 and it should be used. If this is enabled the Value Units from (2) should match the units (mS or S) of the values that were put in the NMRPipe header.
- 4: The Peak File Path button brings up a File-Chooser to select the directory for the peak-pick files. The Peak File Format defines the file names and must include a C-style format starting with % to indicate where the number defining series position is placed.
- 5: The Peak Interpolation button brings up a chooser to define alternate interpolation for the series.
- 6: The Contour Threshold to use for all experiments in the series.
- 7: 'Use Properties' button must be selected in order to make assignments. The button fynSH3 allows defining the sequence and the button fynSH3\_WT allows defining the assignment table.
- 8: The Update All Exps button runs the action to copy common properties defined in this dialog to all experiments in the series. This is optional, it does not have to be clicked. When either the OK or the EditAllExps is selected the copy action is implicitly called only if it has not yet been called for the study. However, after the copy action has been called any changes to a common property require that Update All Exps be clicked to copy the common properties on this dialog to the experiments. The common properties are: Extracted values from header if enabled, Peak File Path, Peak File Format, Peak Interpolation, Contour Threshold, Enable Residue Jump AutoZoom, Initial View Region, Use Properties, Molecule, Assign Table, and Structure.
- 9: The Edit All Exps button brings up a dialog panel to selectively edit properties on individual experiments in the series to allow values different than the common value. The dialog panel allows changing the relaxation delay for each experiment so that if the NMRPipe header does not have the delay set it can be done in this dialog. Before the Edit All Exps dialog is displayed the copy action for Update All Exps is executed if it has not yet been executed so that all experiments are initialized to common.
- 10: Click the Multi-Point Rate button to bring up the Multi-Point Rate Series Model dialog which allows setting formatting and selecting results file.

Clicking OK at the bottom of the Edit Study Dialog will save all of the properties into the file based on the study name, ie twoPointRate.xipp in this example.

## multiPointRate.xipp Series NMR Data File-Set Chooser

The screenshot shows a dialog box titled "Series NMR Data File-Set Chooser". It contains the following fields and controls:

- Project Name:** test%03d.ft2
- Selected File:** test%03d.ft2
- DimCount:** 2
- nmrPipe Label:** HN 15N
- Data Size:** 1038 1024
- 3D/4D File-Set Templates:** Z Format  A Format
- File List:** A list box containing "test%03d.ft2" (highlighted) and a list of files: test001.ft2, test002.ft2, test003.ft2, test004.ft2, test005.ft2, test006.ft2.
- Look In:** A dropdown menu showing "ft" with navigation icons.
- File Name:** An empty text input field.
- Files of Type:** A dropdown menu showing "nmrPipe (\*.DAT, \*.ftxy, \*.ft[1234])".
- Buttons:** "OK" and "Cancel" buttons at the bottom.

This is exactly the same NMR Data File Chooser used to select 2D, 3D or 4D NMR Data. The only difference is that the NMRPipe header for each file indicates that it is a 2D. Do NOT select the 2D files. Select one file-set under 3D/4D File-Set Templates. If no file-sets are shown and the directory is correct modify the Z-Format to match the names of the files. If the file names have a single digit to identify the experiment in the series such as test1.ft2, test2.ft2, test3.ft2, etc then use 1 for the Z Format so that no leading 0 will be put in the file name.

After clicking OK the Multi-Point Study dialog panel will update the Start, End, Peak File Path, Peak File Format and Initial View Region.

## multiPointRate.xipp Peak Pick Options/Interpolation Dialog

- 1: The MatrixVolumeSelector rarely needs to be changed. It defines the number of points that are read in from each experiment every time a peak is picked. The local extrema must be within this volume.
- 2: The Initial Exp. Interpolation defines how the interpolation will be done on the first experiment in the series. This can be set to: '3 Point Parabola', 'Contour Average' or 'Same Position'. The option 'Same Position' should only be used when there are two or more series and this is not the first series.
- 3: The Ensuing Exp. Interpolation defines how the interpolation will be done for all experiments except the first. This can be set to: '3 Point Parabola', 'Contour Average' or 'Same Position'.
- 4: The Average Interpolation in Series for PeakPicks when selected will average the X, Y PPM values using the chosen interpolation method for the experiments and then select the average value as the position for all the PeakPick objects created.

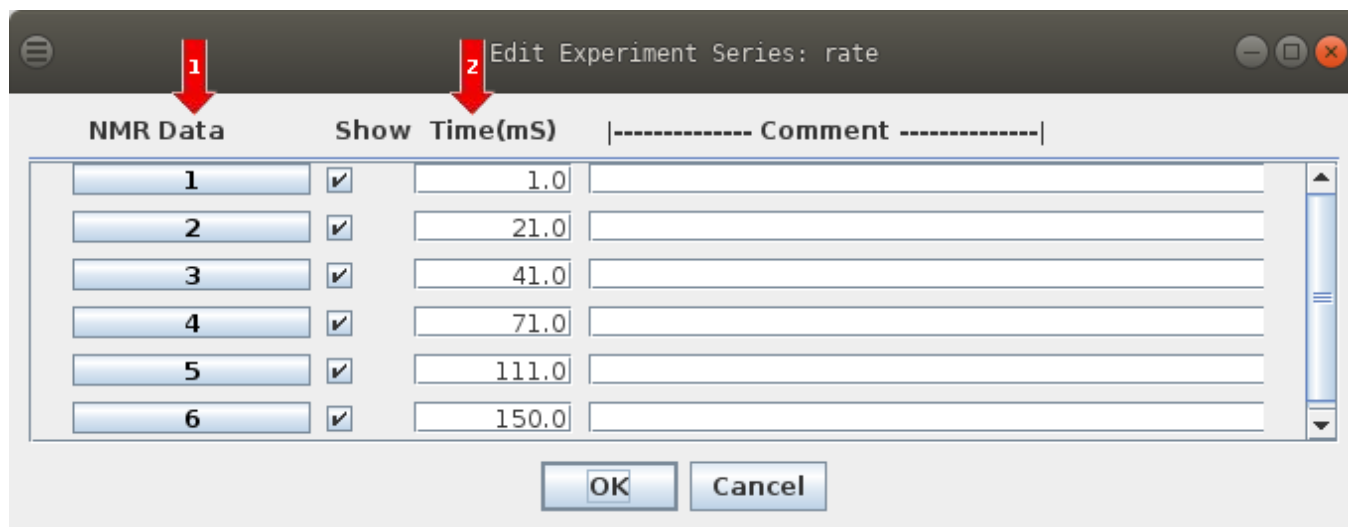
The Study panel Peak Interpolation button text changes to reflect the interpolation choices:

Initial Exp.	Ensuing Exp.	Average Selected	Button text
3 Point Parabola	3 Point Parabola	No	3 Point Parabola
3 Point Parabola	3 Point Parabola	Yes	Average { 3 Point Parabola }
3 Point Parabola	Same Position	No	3 Point Parabola – Same Position
Contour Average	Contour Average	No	Contour Average
Contour Average	Contour Average	Yes	Average {Contour Average }
Contour Average	Same Position	No	Contour Average – Same Position

Other combinations of Initial Exp and Ensuing Exp are possible, but are not as useful. For example 'Average { 3 Point Parabola – Same Position } gives the same peak-pick positions as 3 Point Parabola – Same Position.



## multiPointRate.xipp Edit All Exps

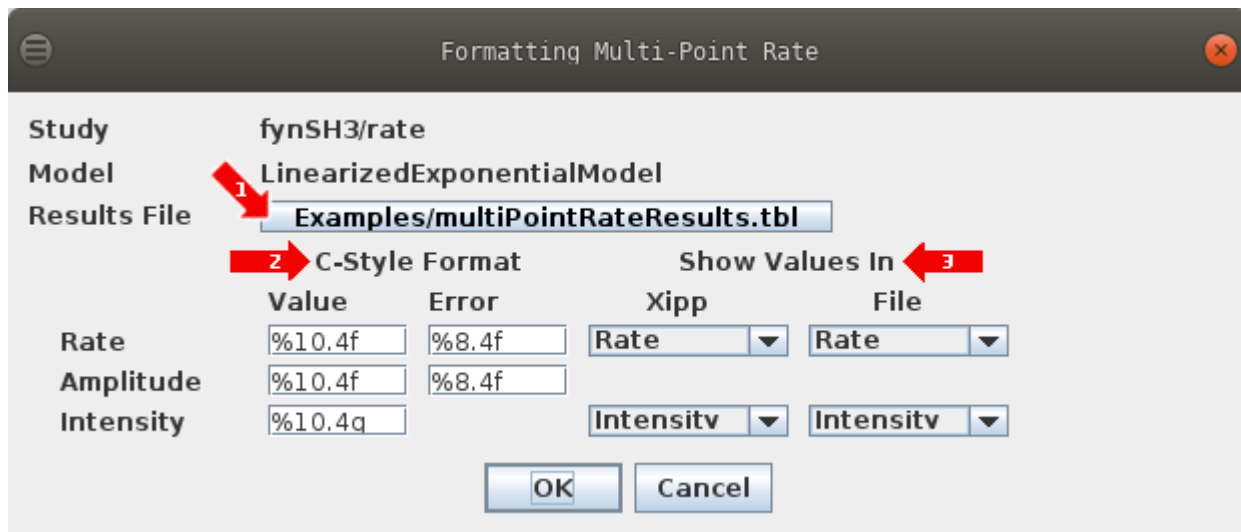


1: Click one of the NMR Data buttons to bring up the Edit NMR Experiment Dialog to modify experiment properties. This is the same Edit NMR Experiment Dialog that has been described several times and will not be described again.

2: If needed enter or update the relaxation time. Note the units mS are set on Study Dialog (2) via Value Units which can be mS or S. The list of relaxation delay values are also shown in the results file.

Click OK at the bottom of the Dialog to save all of the experiment properties.

## multiPointRate.xipp Multi-Point Rate Series Model



- 1: Click the Results File button to bring up the File Chooser to select the results file.
- 2: Enter new C-Style format if different format as needed.
- 3: The 'Show Values In' buttons for the Multi-Point Rate study currently have only one option except for Intensity in File which can be Intensity or None.

Click OK at the bottom of the Dialog to save the series model properties.

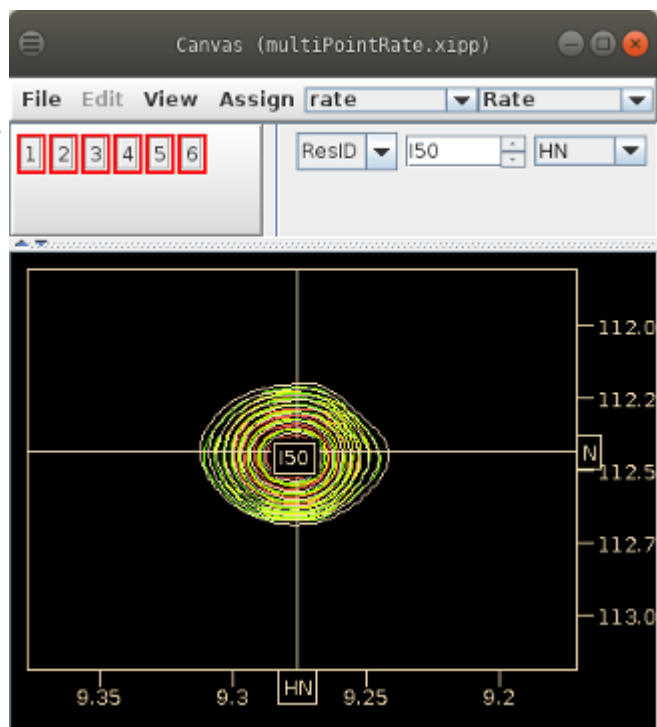
## multiPointRate.xipp Show Study

Ensure that multiPointRate (fynSH3) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the Multi-Point Rate Study.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (2) Enter Keyboard command: Alt-J 50  
to residue jump I50. You can also type 50 into Jump window and hit enter.
- (3) Click left mouse button on peak at 9.3, 112.5 PPM to Peak-Pick and assign I50.

The contours for experiments 1 through 6 are shown in the Canvas Window.



The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The bottom part always shows information about peaks that have been picked. The slider between the top part and bottom part has been moved up so that only I50 N and HN are shown. As peak-picks are created they are added to the top of the Name-Peak Table in the bottom part of the Table Window. The Name-Peak Table shows the peak-picks from experiments 6, 5 and 4. The peak-picks from experiments 3, 2 and 1 are accessible via the scroll-bar. Prior to taking a screenshot the Table and Canvas windows were reduced by almost half so that the text would be easier to read. At normal size six HSQC peak-picks can be seen at once before using a scroll-bar.

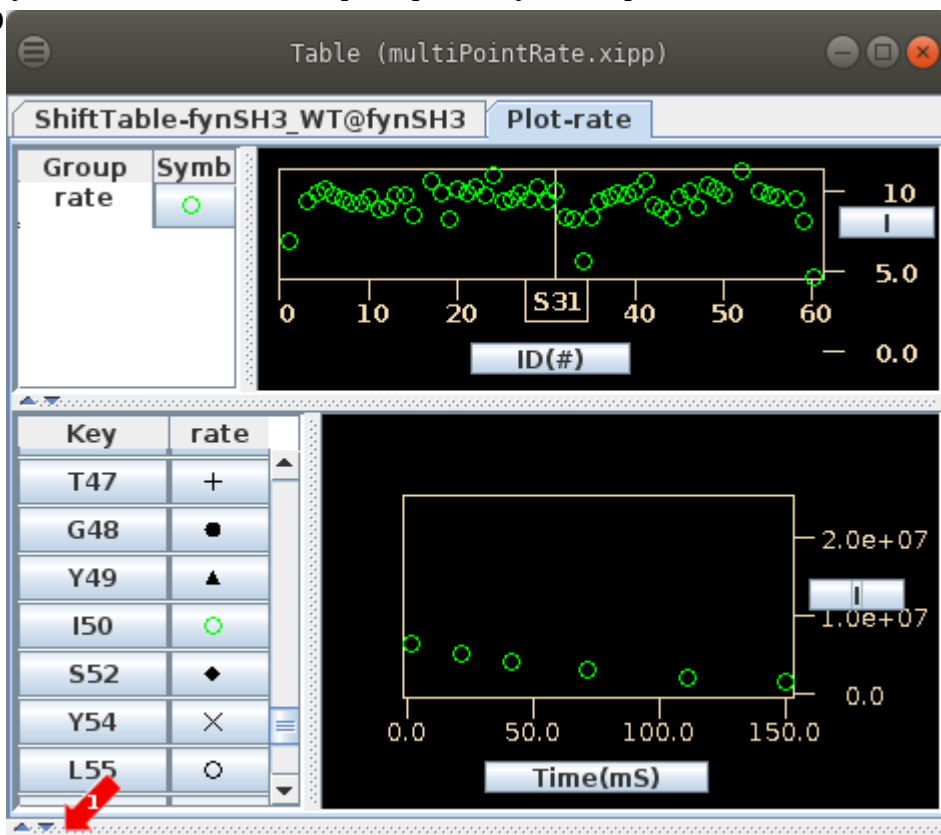
Residue	AtomID	AtomName	Shift	RMS	Count
I50+	10852-	N	112.4320	-	-
	10853-	HN	9.2760	-	-

Contours	PkID	Assign	HN	N	I
	1	I50.N,HN	9.277	112.448	1.471e+6
	K+	6 Assigns (1)	HN	N	
		I50.N,HN	-0.0006	-0.0155	
	1	I50.N,HN	9.278	112.452	2.138e+6
	K+	5 Assigns (1)	HN	N	
		I50.N,HN	-0.0018	-0.0198	
	1	I50.N,HN	9.277	112.452	3.131e+6
	K+	4 Assigns (1)	HN	N	
		I50.N,HN	-0.0013	-0.0195	

## multiPointRate.xipp Rate Table Plot/File

CAPP should not be used with any Series data since it will peak-pick only one experiment at a time and will not ensure the same peak ID between experiments. Manual peak-picking for the Multi-Point Rate Study was done in exactly the same way as in the Two-Point Rate Study. First Alt-J to S1 to create the S1 peak-pick and then using Alt up-arrow key to jump to the next residue to peak-pick, ie from S1 to R60. For each residue a single mouse click peak-picks all six experiments, assigns each peak-pick, updates the plots and results file. The Plot-Rates tab of the Table Window after manually peak-picking and assigning residues T2 to R60 is shown at the right. Note the Rate is shown at top for residues that have been assigned and the bottom shows the intensity decay for I50. The Name Peak-Pick Table has been hidden, but can be shown by moving the divider at (1). The little triangles can be used to quickly change the divider location.



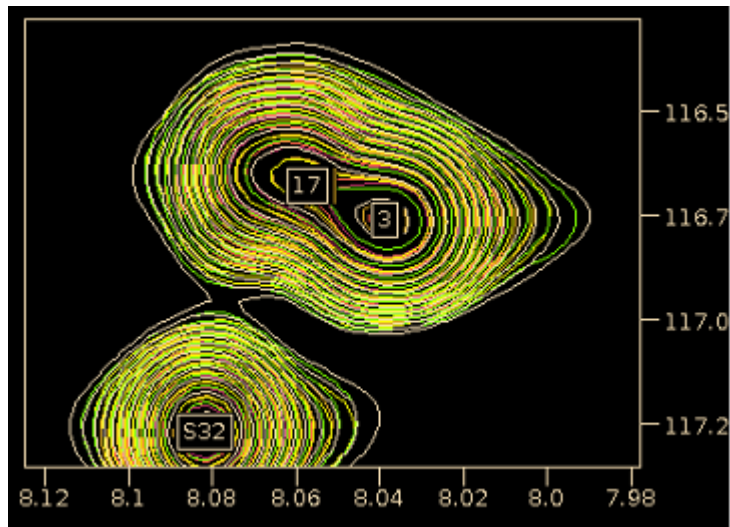
The results file multiPointRateResults.tbl is automatically created and updated while the experiments are peak-picked. Here is a portion of the results file (in 10 point font) showing the tabulated data:

```

VARS  KEY RATE ERROR AMPLITUDE ERROR INTENSITY_1 INTENSITY_2 INTENSITY_3
INTENSITY_4 INTENSITY_5 INTENSITY_6
FORMAT %4d %10.4f %8.4f %10.4f %8.4f %10.4g %10.4g %10.4g %10.4g %10.4g
#
# Time(S): 0.001000 0.02100 0.04100 0.07100 0.1110 0.1500
# Key Rate Error I(Eq) Error I(1) I(2) I(3) I(4) I(5) I(6)
1 6.9132 0.1154 13191830.4749 0.0097 1.324e+07 1.150e+07 9.835e+06 7.900e+06 6.169e+06
4.708e+06
2 7.5248 0.0512 15502340.4409 0.0043 1.549e+07 1.319e+07 1.134e+07 9.039e+06 6.779e+06
5.004e+06
3 9.4383 0.1047 11104795.9299 0.0088 1.099e+07 8.974e+06 7.639e+06 5.697e+06 3.945e+06
2.663e+06
4 9.9389 0.1478 7284880.4889 0.0124 7.350e+06 5.864e+06 4.841e+06 3.561e+06 2.368e+06 1.676e+06
5 10.1236 0.2132 7870386.5865 0.0178 7.699e+06 6.375e+06 5.196e+06 3.830e+06 2.669e+06
1.671e+06

```

## multiPointRate.xipp Assigning Ambiguous



Fixing ambiguous assignments in a Multi-Point Rate study requires selecting the correct assignment for each of the six experiments. Click the left mouse button on the Canvas Window over peak ID 3 to select peak ID 3 which should the peak-picks from experiments 1, 2, 3, 4, 5 and 6 at the top of the Name-Peak Table. On the Table Window select T2 as the assignment for all six experiments. You should not have to scroll the table to see the six peak-picks for peak ID 3. The Table Window shown below is the same Table Window above with the same size. The divider (1) is moved all the way to the top by clicking the solid up triangle in the divider. The peak-picks from experiments 3, 2 and 1 are visible in the reduced size Table Window. The experiment name for each peak-pick is shown before the Assignment column header (2).

PKID	Assign	HN	N	Intensity
<b>Assignment-Table</b>				
3	T2.N,HN	8.039	116...	1.134e+7
K+	3 Assigns (2)	HN	N	
	T2.N,HN	-0.0018	-0.0151	
	D16.N,HN	0.0202	-0.1221	
3	T2.N,HN	8.039	116...	1.319e+7
K+	2 Assigns (2)	HN	N	
	T2.N,HN	-0.0018	-0.0142	
	D16.N,HN	0.0202	-0.1212	
3	T2.N,HN	8.039	116...	1.549e+7
K+	1 Assigns (2)	HN	N	
	T2.N,HN	-0.0019	-0.0153	
	D16.N,HN	0.0201	-0.1223	

Do the same for peak ID 17. Since peak ID 3 is more intense the mouse should be placed above and to the left of peak ID 17 so that when Xipp looks for a local extrema it will find peak ID 17 not 3.

## pre.xipp Two-Point PRE Study

The Two-Point PRE and Two-Point Delta Rate uses the same dialog and differ by selecting PRE or Delta-R2 in option (1) and having different Series Model in (6).

The screenshot shows the 'Edit Study: pre' dialog box with the following settings and annotations:

- Startup File:** pre
- Study:** HPr/pre
- Comment:** (empty)
- Series Type:** Two-Point **PRE** (arrow 1)
- NMR Data Exps:**
  - At Time(t1):** Diagmagnetic **Dia t1** (arrow 2)
  - At Time(t2):** Diagmagnetic **Dia t2**
  - Rate Models:** **Two-Point Rate**
- Paramagnetic:**
  - Para t1** (arrow 3)
  - Para t2**
  - Rate Models:** **Two-Point Rate**
- delta T = t2 - t1:** mS, 36 (arrow 4)
- Same delta-T:**  36 (arrow 5)
- Peak Interpolate:** 3 Point Parabola
- Enable Residue Jump AutoZoom:**
- Initial View Region:**
  - Set Region by:**  Calc Union  Calc Overlap  Manual
  - Display-X:** HN (arrow 6)
  - Display-Y:** N
  - User Origin:** 10.997
  - User End:** 5.994
- Use Properties:**  **Same Properties:**
- Molecule:** HPr
- Assign Table:** hprAssign
- Structure:** Set Structure
- Series Model:** **Two-Point PRE** (arrow 6)
- Results in File:** Examples/preResults.tbl
- Buttons:** OK, Cancel

## pre.xipp Two-Point PRE Study

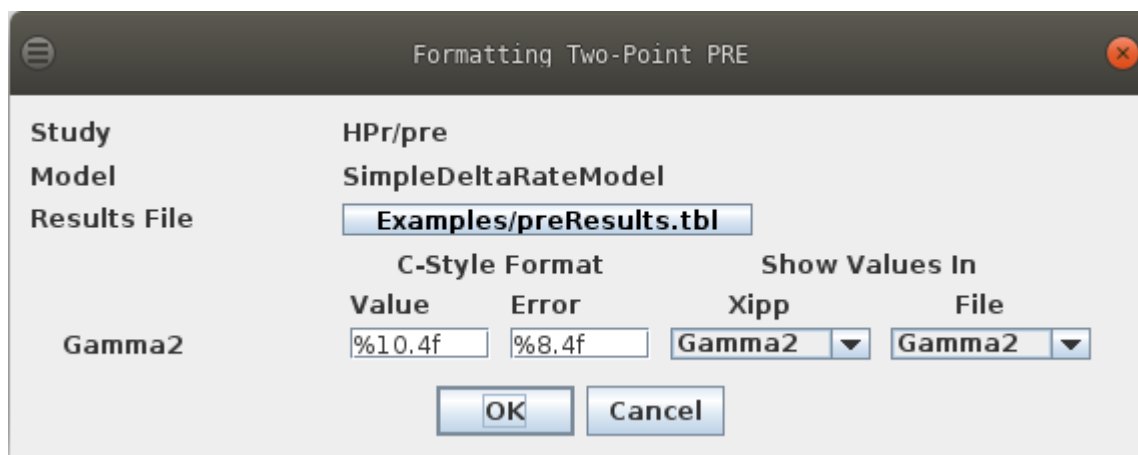
1: The Series Type is fixed to Two-Point and the Sub-Type can be set to PRE or Delta-R2. As far as Xipp as concerned the difference between a PRE and a delta-rate study is semantics as they both involve the difference in rate and involves different labels and names. Changing the Sub-Type changes the experiment names, Series Model and name of results file. Changes to Series Model in 6 followed by changing Series Sub-Type will result in loss of values entered for Series Model. The Series Model properties may get set to default values when series type or sub-type change.

2: Click buttons Dia\_t1 and Dia\_t2 to bring up the Experiment Dialog at time t1 or t2 respectively to define the file names and properties of the 2D  $^{15}\text{N}$  HSQC NMR experiments for the Diamagnetic experiments. Note the text on the buttons will be red when the properties are not correctly set. The Two-Point Rate button below the Dia\_t1 and Dia\_t2 buttons brings up the Two-Point Rate Series Model for diamagnetic sample.

3: Click buttons Para\_t1 and Para\_t2 to bring up the Experiment Dialog at time t1 or t2 respectively to define the file names and properties of the 2D  $^{15}\text{N}$  HSQC NMR experiments for the Paramagnetic experiments. Note the text on the buttons will be red when the properties are not correctly set. The Two-Point Rate button below the Para\_t1 and Para\_t2 buttons brings up the Two-Point Rate Series Model for paramagnetic sample.

4 & 5: Enter the diamagnetic and paramagnetic relaxation delay time. When Same delta-T is enabled the diamagnetic relaxation delay is used for both samples.

6: Click Two-Point PRE button to change Series Model properties which include location of Results File and formatting specification.

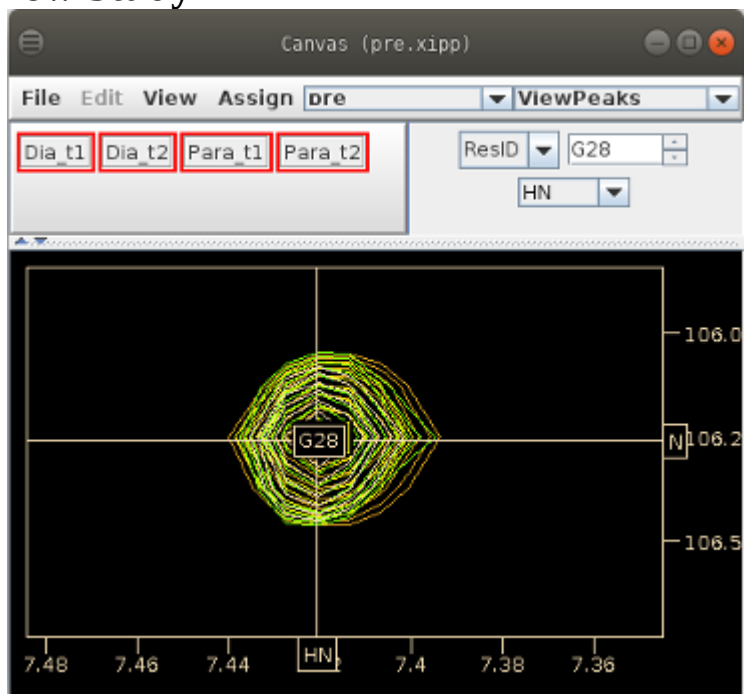


## pre.xipp Show Study

Ensure that pre (HPr) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the Two-Point PRE Study.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (2) Enter Keyboard command: Alt-J 28 to residue jump G28. You can also type 28 into Jump window and hit enter.
- (3) Click left mouse button on peak at 7.4, 106.2 PPM to peak-pick and assign G28.



The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The bottom part always shows information about peaks that have been picked. As peak-picks are created they are added to the top of the Name-Peak Table. The Dia\_t1 peak-pick was added first and is shown at the bottom with the peak-pick. The Para\_t2 peak-pick was added second and is at the top of the Name-Peak Table. The color of the contours in the Name-Peak Table match the color in Canvas Window for the same experiment.

Residue	Atom-Assignment-Table				
	AtomID	AtomName	Shift	RMS	Count
G28+	10490-	N	106.2550	-	-
	10491-	HN	7.4210	-	-

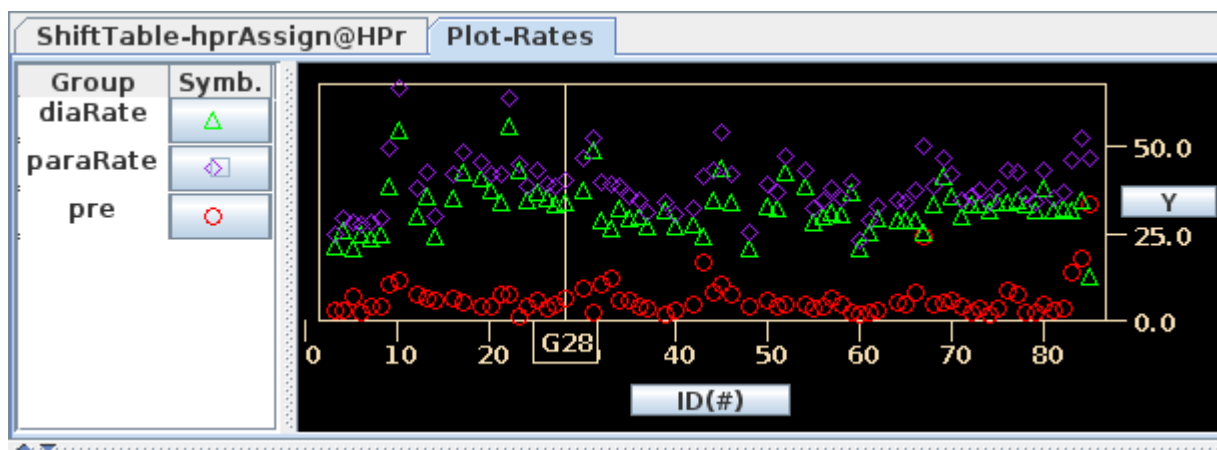
  

Contours	PkID	Assign	HN	N	Intensity
	1	G28.N,HN	7.419	106.247	2.847e+6
	K+	Para_t2 Assigns (1)		HN	N
		G28.N,HN	0.0019	0.0082	
	1	G28.N,HN	7.420	106.249	1.228e+7
	K+	Para_t1 Assigns (1)		HN	N
		G28.N,HN	0.0014	0.0060	
	1	G28.N,HN	7.421	106.251	3.217e+6
	K+	Dia_t2 Assigns (1)		HN	N
		G28.N,HN	0.0005	0.0036	
	1	G28.N,HN	7.421	106.254	1.094e+7
	K+	Dia_t1 Assigns (1)		HN	N
		G28.N,HN	0.0004	0.0009	



## pre.xipp Rate Table Plot/File

CAPP should not be used with any Series data since it will peak-pick only one experiment at a time and will not ensure the same peak ID between experiments. Manual peak-picking for the Multi-Point Rate Study was done in exactly the same way as in the Two-Point Rate Study. First Alt-j to Q3 to create the Q3 peak-pick and then using Alt up-arrow key to jump to the next residue to peak-pick, ie sequentially from Q3 to E85. For each residue a single mouse click peak-picks all four experiments, assigns each peak-pick, updates the plots and results file. The Plot-Rates tab of the Table Window after manually peak-picking and assigning is shown below.



The results file preResults.tbl is automatically created and updated while the experiments are peak-picked. Here is a portion of the results file showing the tabulated data:

```

VARS  KEY Gamma2 ERROR R2_DIA R2_DIA_ERR R2_PARA R2_PARA_ERR
FORMAT %4d %10.4f %8.4f %10.4g %8.4f %10.4g %8.4f

```

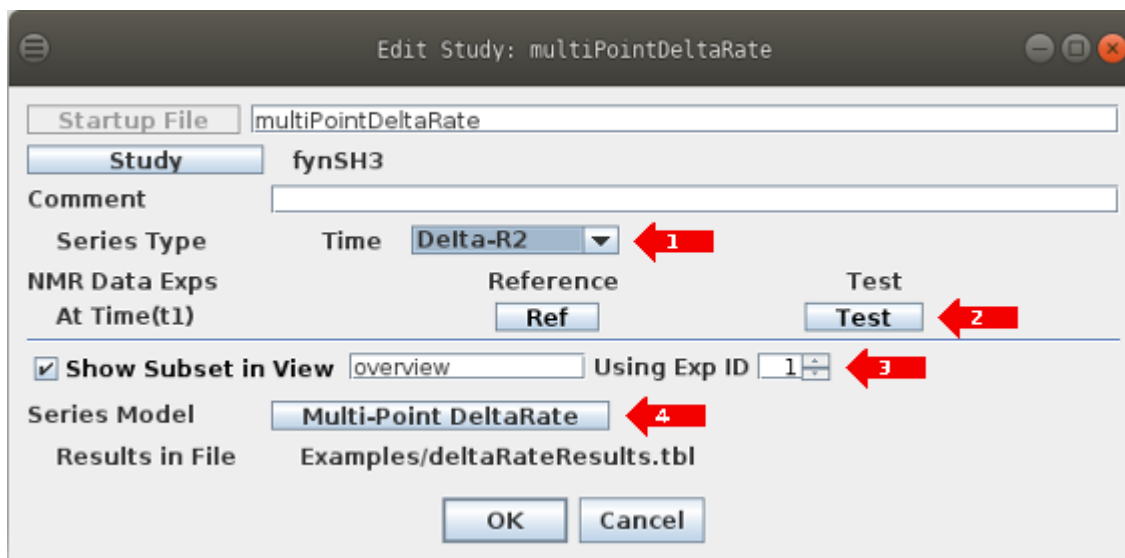
#	Key	Value	Error	R2(Dia)	Err(Dia)	R2(Para)	Err(Para)
3	3.1968	0.0000	21.76	0.0000	24.95	0.0000	
4	3.3808	0.0000	26.19	0.0000	29.58	0.0000	
5	7.2064	0.0000	21.31	0.0000	28.51	0.0000	
6	2.8252	0.0000	25.41	0.0000	28.23	0.0000	
7	4.3851	0.0000	24.22	0.0000	28.60	0.0000	
8	4.4893	0.0000	25.19	0.0000	29.67	0.0000	
9	10.7341	0.0000	38.72	0.0000	49.46	0.0000	
10	11.9282	0.0000	54.80	0.0000	66.72	0.0000	
12	7.9955	0.0000	30.08	0.0000	38.08	0.0000	

The column labeled Key is the residue number.

## multiPointDeltaRate.xipp

### Multi-Point Delta Rate Study

The Multi-Point PRE and Multi-Point Delta Rate uses the same dialog and differ by selecting PRE or Delta-R2 in option (1) and having different Series Model in (4).



1: The Series Type is fixed to Time and the Sub-Type can be set to PRE or Delta-R2. As far as Xipp is concerned the difference between a PRE and a delta-rate study is semantics as they both involve the difference in rate. Changing the Sub-Type changes the experiment names, Series Model and name of results file. Changes to Series Model in (4) followed by changing Series Sub-Type will result in loss of values entered for Series Model. The Series Model properties may get set to default values when series sub-type is changed.

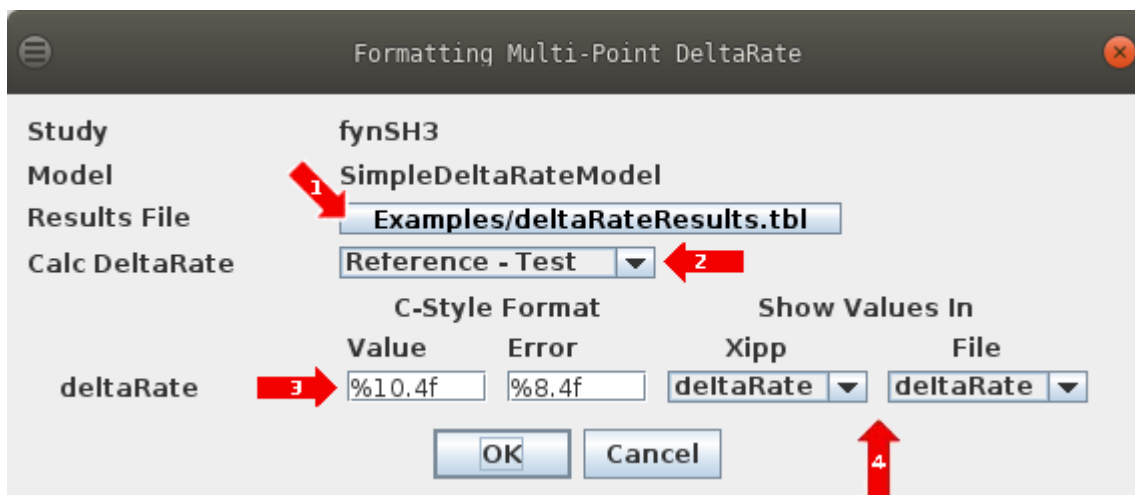
2: Click buttons Ref and Test to bring up the Experiment Series Dialog for the reference and test Multi-Point Rate experiments. See the previously described Multi-Point Rate Study for a description of the Experiment Series Dialog that is displayed when either the Ref or Test button is clicked. In fact the previously described Multi-Point Rate Study is the Ref in this Study.

3: By default Show Subset in View is enabled that will put the first 2D  $^{15}\text{N}$  HSQC experiment from Ref and Test series in a separate View named overview. The view name and which experiment to use can be changed.

4: Click Multi-Point DeltaRate button to change Series Model properties which includes location of Results File and formatting specification.

Clicking OK at the bottom of the Edit Study Dialog will save all of the properties into the file based on the study name, ie multiPointDeltaRate.xipp in this example.

## multiPointDeltaRate.xipp Multi-Point Delta Rate Series Model



- 1: Click the Results File button to bring up the File Chooser to select the results file.
- 2: Set how delta is calculated if needed.
- 3: Enter new C-Style format if different format as needed.
- 4: The 'Show Values In' buttons for the Multi-Point Delta Rate study currently have only one option. The intensities for the Ref and Test Series can be recorded in refResults.tbl and TestResults.tbl respectively which is set in the Multi-Point Rate Series Model dialogs for Ref and Test Experiment Series.

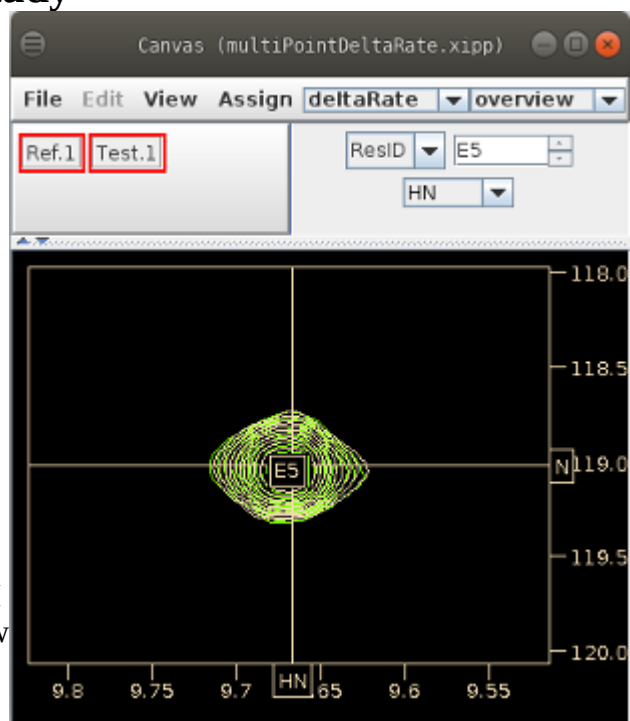
Click OK at the bottom of the Dialog to save the series model properties.

## multiPointDeltaRate.xipp Show Study

Ensure that multiPointDeltaRate (fynSH3) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the Multi-Point Delta Rate Study.

At right are the Canvas and Table windows after entering the following commands and mouse clicks on the Canvas:

- (1) Keyboard command: n  
To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data
- (2) Enter Keyboard command: Alt-j  
to residue jump E5. You can also type 5 into Jump window and hit enter.
- (3) Click left mouse button on peak at 9.7, 119.0 PPM to Peak-Pick and assign E5. Peak-picking in overview will forward peak-picks to Ref and Test Experiment Series so that 12 peak-picks are created.



The default View is overview showing experiments 1 from the Ref and Test series in the Canvas Window.

The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The bottom part shows information about peaks that have been picked. The Name-Peak Table has 12 peak-picks (six from each series). The scroll-bar is shifted down so that the E5 peak-picks for experiment 1 from Test and experiments 6 and 5 from Ref are shown. Normally the Name-Peak Table shows six peak-picks with default size of the Table Window. Unfortunately, only the experiment name excluding Ref and Test are shown so it is difficult to distinguish.

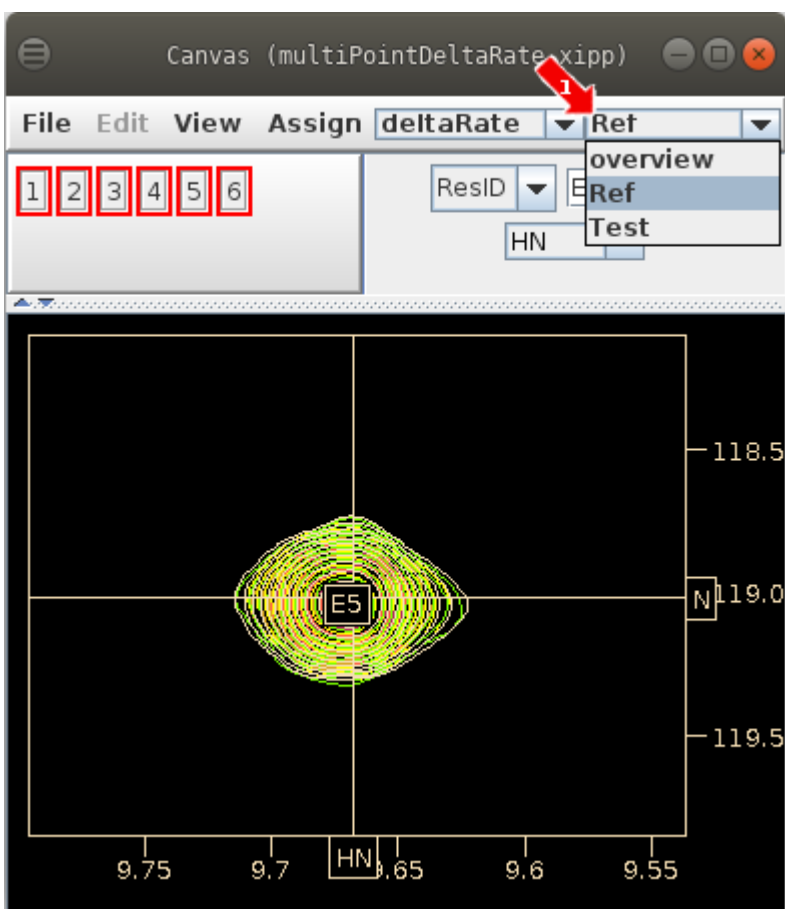
Residue	Atom-Assignment-Table				
	AtomID	AtomName	Shift	RMS	Count
150+	10852-	N	112.4320	-	-
	10853-	HN	9.2760	-	-

Contours	PKID	Assign	HN	N	Inte
	1	E5.N,HN	9.669	119.038	8.627e+6
	K+	1 Assigns (1)	HN	N	
		E5.N,HN	-0.0009	-0.0227	
	1	E5.N,HN	9.671	119.038	1.671e+6
	K+	6 Assigns (1)	HN	N	
		E5.N,HN	-0.0028	-0.0235	
	1	E5.N,HN	9.671	119.038	2.667e+6
	K+	5 Assigns (1)	HN	N	
		E5.N,HN	-0.0028	-0.0235	

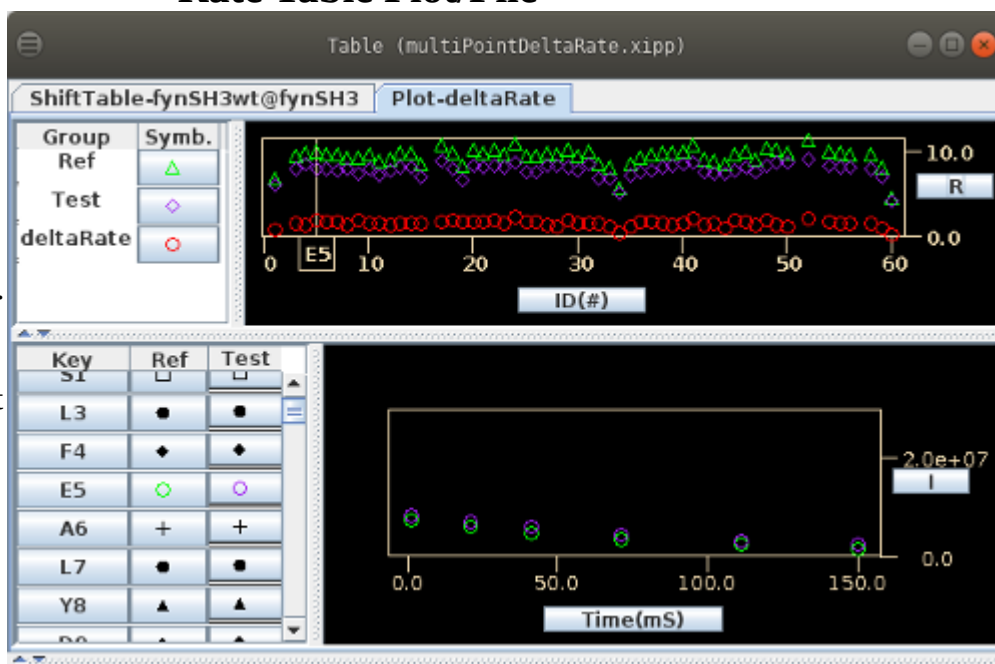
## multiPointDeltaRate.xipp Select Ref View

The Multi-Point Delta Rate is difference between two Multi-Point Rates. Adding/deleting peak-picks in the overview automatically adds/deletes peaks in both the Ref and Test experiment series. Use the View Chooser (1) to select either the Ref or Test samples to see the spectra for all experiments in either series. Note peak-picking in the Ref or Test view will only add/delete peaks in the selected series such as Ref in this case.



## multiPointDeltaRate.xipp Rate Table Plot/File

The CAPP dialog will not open when used in the overview. Manual peak-picking for the Multi-Point Delta Rate Study was done in exactly the same way as in the Two-Point Rate Study. In the overview View first Alt-j to S1 to create the S1 peak-pick and then using Alt up-arrow key to jump to the next residue to peak-pick, ie from S1 to R60. For each residue a single mouse click peak-picks all 12 experiments, assigns each peak-pick, updates the plots



and the results file. The Plot-deltaRate tab of the Table Window after manually peak-picking and assigning residues T2 to R60 is shown at the right. Note the Rate is shown at top for residues that have been assigned and the bottom shows the intensity decay for I50. The Name Peak-Pick Table has been hidden by moving the divider to the bottom as was done in the Multi-Point Rate Study. Color of the symbol in the two tables indicates whether data is from Ref or Test. The same symbol shape for same residue is used in lower plot to match data from same residue, but symbols get re-used.

Three results files are created and automatically updated: refResults.tbl, testResults.tbl and deltaRateResults.tbl. Here is a portion of the deltaRateResults.tbl:

```

VARS  KEY DeltaRate ERROR R2_REF R2_REF_ERR R2_TEST R2_TEST_ERR
FORMAT %4d %10.4f %8.4f %10.4g %8.4f %10.4g %8.4f

```

#	Key	Value	Error	R2(Ref)	Err(Ref)	R2(Test)	Err(Test)
1		0.8018	0.1369	6.913	0.1155	6.112	0.0735
3		1.5288	0.1357	9.438	0.1047	7.909	0.0862
4		1.3839	0.2001	9.926	0.1504	8.542	0.1321
5		2.0673	0.2717	10.13	0.2100	8.059	0.1725
6		1.7427	0.0874	9.727	0.0803	7.984	0.0347
7		1.7358	0.1523	9.571	0.1285	7.836	0.0817
8		1.3971	0.0769	9.311	0.0632	7.914	0.0438
9		1.9114	0.0576	9.312	0.0276	7.400	0.0506
10		1.6694	0.1977	9.623	0.1751	7.954	0.0916
11		1.6408	0.0936	8.880	0.0702	7.239	0.0620
12		1.3343	0.0990	9.032	0.0666	7.698	0.0732
13		1.6231	0.2668	9.814	0.2259	8.191	0.1420

# CPMG\_Dispersion.xipp

## Edit Study

The screenshot shows the 'Edit Study: CPMG Dispersion' dialog box. The window title is 'Edit Study: CPMG Dispersion'. The 'Startup File' is 'CPMG Dispersion'. The 'Study' is 'fynSH3/rate'. The 'Comment' field is empty. The 'NMR Data Series' is 'test%03d.ft2', with 'Start' set to 1 and 'End' to 25. The 'Default Exp. Type' is '15N-HSQC-NoNew'. The 'Series Type' is 'Tau' (indicated by red arrow 2) and the sub-type is 'Disperse' (indicated by red arrow 3). The 'Value Units' are 'Hz'. There is a checked box for 'Extract value from NMRPipe' with a value of 199. The 'dt = t2 - t1' is 'mS' with a value of 33 (indicated by red arrow 4). The 'Peak File Path' is 'proc/ft' (indicated by red arrow 4) and the 'Peak File Format' is 'test%03d' (indicated by red arrow 5). The 'Peak Interpolation' is 'Average { 3 Point Parabola }' (indicated by red arrow 6). The 'Contour Threshold' has a 'Level' of 3.500e+05, a 'Multiplier' of 1.3, and 'Calculate' set to 'All'. There is a checked box for 'Enable Residue Jump AutoZoom'. The 'Initial View Region' is set by 'Calc Union'. The 'Axis Name' are 'HN' (Display-X) and 'N' (Display-Y). The 'User Origin' is (10.2, 131.791) and the 'User End' is (6.497, 107.129). There is a checked box for 'Use Properties'. The 'Molecule' is 'fynSH3', the 'Assign Table' is 'fynSH3', and the 'Structure' is 'Set Structure' (indicated by red arrow 7). At the bottom, there are two buttons: 'Update All Exps' (indicated by red arrow 7) and 'EditAllExps' (indicated by red arrow 8). The 'Series Model' is 'Disperse Series' (indicated by red arrow 9) and the 'Results in File' is 'Examples/rateResults.tbl'. 'OK' and 'Cancel' buttons are at the bottom.

The Experiment Series Study allows the creation of a variety of Experiment Series based on a series of 2D <sup>15</sup>N-HSQC spectra. Shown here is a CPMG Dispersion series with 25 2D <sup>15</sup>N-HSQC experiments. Selecting Series Type (2) a Tau and sub-type Disperse makes it a CPMG Dispersion.

Note the text on a button will be red when the properties accessed by that button are not correctly set.

## CPMG\_Dispersion.xipp

### Edit Study

This dialog defines one CPMG Dispersion Experiments Series Study with 25 <sup>15</sup>N-HSQC NMR experiments.

**1:** The 'NMR Data Series' button is used to bring up the Series NMR Data File-Set Chooser Dialog to select the series of 2D NMR experiments. Note the file names must include a number in sequential order such as test001.ft2, test002.ft2, test003.ft2, . . . test024.ft2 and test025.ft2 so that Xipp can identify the series using a simple name such as test%03d.ft2 which will be used as the text on this button.

**2:** The Series Type button determines the type of Series. This can be set to Series, Time or Tau.

**3:** The button next to Series Type is the Series sub-type and its allowed settings depend on the selection for Series Type. For Series only Generic is allowed for sub-type. For Time the sub-type can be Generic, T1, T1p or T2. For Tau the sub-type can be Generic or Disperse. Changing the Series Type and sub-type automatically changes the 'Series Model'.

**4:** The Peak File Path button brings up a Peak-Pick File chooser to select a directory for the Peak-Pick files. Each 2D experiment will have its own Peak-Pick file. By default this is set to the same directory as the NMR data was located.

**5:** The Peak File format defines the format for the name for each Peak-Pick file and by default is the same as the file name for the NMR experiments excluding the file extension. The Peak-Pick file names all have .ASG and .PCK as their extension so the Peak-Pick file names will not overwrite the NMR data file names (unless of course you choose to name your experiments test%03d.ASG which would cause the Peak-Pick file to overwrite the NMR Data data file).

**6:** The 'Peak Interpolation' button brings up the Peak Pick Options dialog to select type of interpolation on Initial Experiment and Ensuing Exps.

**7:** The 'Update All Exps' button will copy the properties on this dialog to each 2D Experiment. When the Study is first created and the OK button is clicked all 2D experiments are automatically updated. After the Study has been created and saved and when it is edited any changes to Peak File Path/Format, Contour Threshold, Initial View Region and Use Properties (Molecules & Assign Table) will not automatically update to each 2D NMR Experiment unless the Update All Exps is clicked.

**8:** The 'Edit All Exps' button brings up the Edit Experiment Series dialog that allows setting properties for individual Exps. This should not be needed under normal situations, but exists to provide the flexibility to set properties differently for individual experiments. The Edit Experiment Series dialog also provides a way to set and confirm the Tau value for each experiment if this was not set when the data was processed by NMRPipe.

**9:** The 'Series Model' button brings up the Model dialog which allows setting the Results files and formatting.



## CPMG\_Dispersion.xipp

### Show Study: Canvas

Ensure that CPMG\_Dispersion (fynSH3) is selected (ie highlighted) on the XippPanel. Click the Show button to display the Canvas and Table windows for the CPMG Dispersion Study.

Below and on the next page are the Canvas and Table Windows after entering the following commands and mouse clicks on the Canvas:

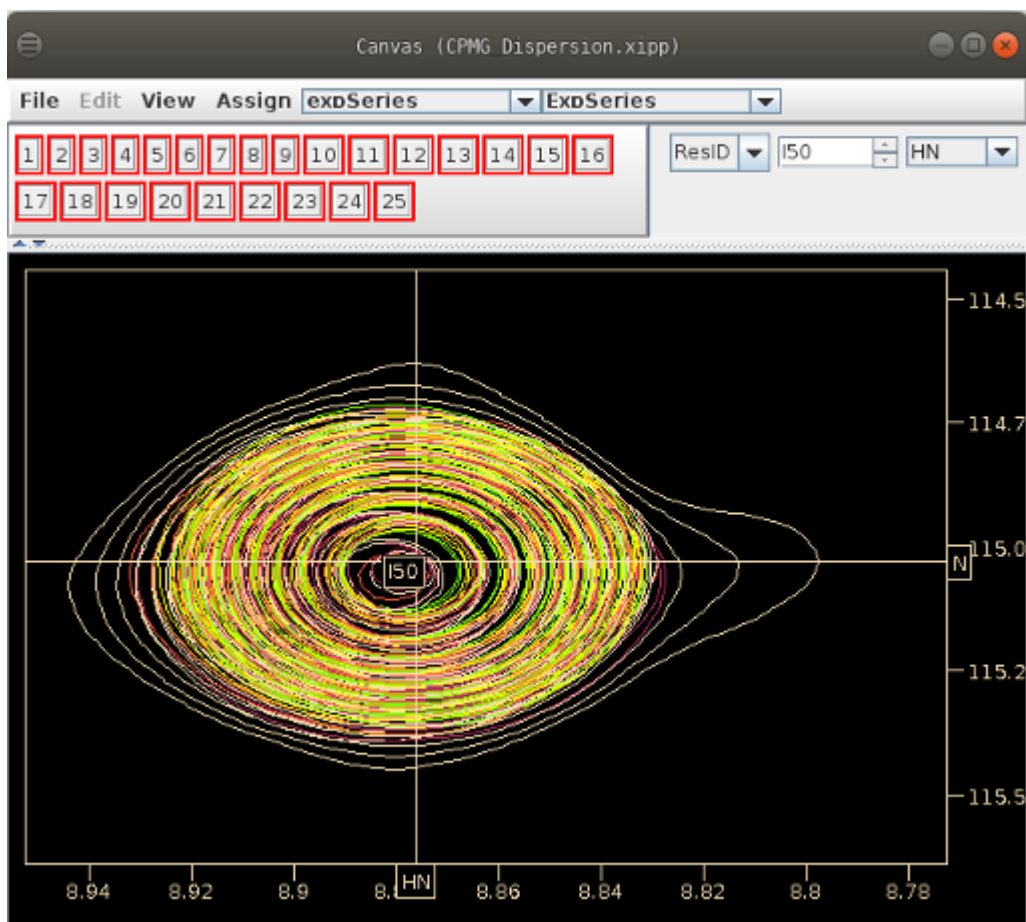
(1) Keyboard command: n

To toggle to Name/Assign Peak Table in the Table Window. n can be entered anywhere in the canvas window with the NMR Data

(2) Enter Keyboard command: Alt-j 50

to residue jump I50. You can also type 50 into Jump window and hit enter.

(3) Click left mouse button on peak at 8.9, 115.0 PPM to Peak-Pick and assign I50. Peak-picking in overview will forward peak-picks to Ref and Test Experiment Series so that 12 peak-picks are created.




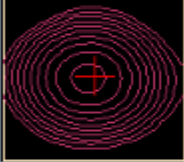



## CPMG\_Dispersion.xipp

### Show Study: Table

The top part of the Table Window is tabbed and shows the ShiftTable or a plot. The ShiftTable in the top part shows the assignments for I50. The bottom part shows information about peaks that have been picked. The Name-Peak Table has 25 peak-picks. The scroll-bar is at the top so that the I50 peak-picks from experiments 25, 24, 23, 22 and 21 are shown. Normally the Name-Peak Table shows six peak-picks with default size of the Table Window. Resolving ambiguous peaks is more difficult since each peak-pick in the Name-Peak Table must be individually changed.

Residue		Atom-Assignment-Table			
	AtomID	AtomName	Shift	RMS	Count
I50+	10852-	N	115.0284	-	-
	10853-	HN	8.8765	-	-

Contours	PkID	Assign	HN	N	Intensit
	Assignment-Table				
	1	I50.N,HN	8.879	115.047	4.236e+6
	K+	25 Assigns (1)	HN	N	
		I50.N,HN	-0.0025	-0.0185	
	1	I50.N,HN	8.879	115.047	4.102e+6
	K+	24 Assigns (1)	HN	N	
		I50.N,HN	-0.0025	-0.0185	
	1	I50.N,HN	8.879	115.047	3.912e+6
	K+	23 Assigns (1)	HN	N	
		I50.N,HN	-0.0025	-0.0185	
	1	I50.N,HN	8.879	115.047	3.709e+6
	K+	22 Assigns (1)	HN	N	
		I50.N,HN	-0.0025	-0.0185	
	1	I50.N,HN	8.879	115.047	3.549e+6
	K+	21 Assigns (1)	HN	N	
		I50.N,HN	-0.0025	-0.0185	

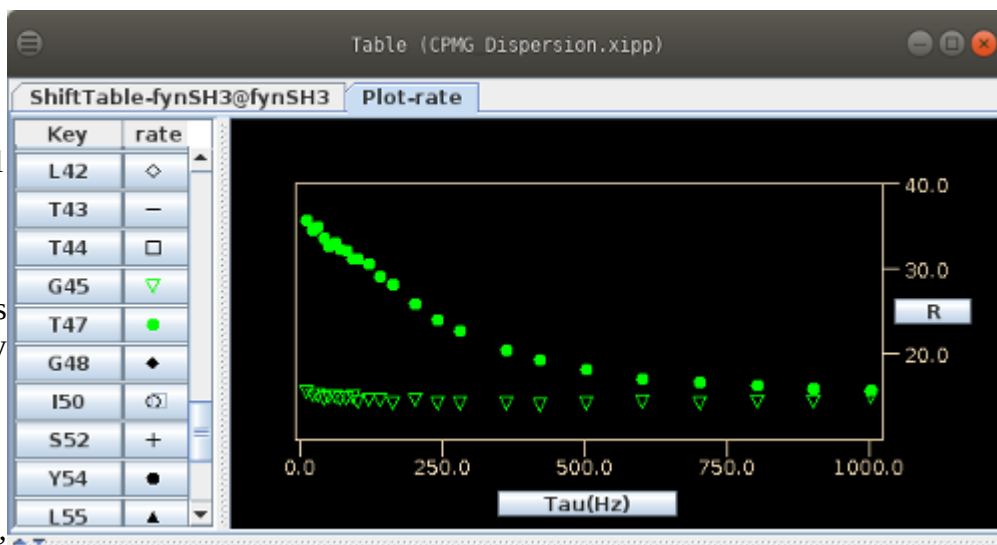
## CPMG\_Dispersion.xipp Rate Table Plot/File

CAPP should not be used with any Series data since it will peak-pick only one experiment at a time and will not ensure the same peak ID between experiments.

Manual peak-picking for the CPMG Dispersion Study was done in exactly the same way as in the previous studies.

First Alt-j to S1 to create the S1 peak-pick and then using Alt up-arrow key to jump to the next residue to peak-pick, ie from S1 to R60. For each

residue a single mouse click peak-picks all 25 experiments, assigns each peak-pick, updates the plot and the results file. The Plot-rate tab of the Table Window after manually peak-picking and assigning residues T2 to R60 is shown above. The Name Peak-Pick Table is not shown.



The results file rateResults.tbl is automatically created and updated while the experiments are peak-picked. Here is a portion of the results file (in 10 point font) showing the tabulated data:

```

VARS KEY INTENSITY_0 INTENSITY_1 INTENSITY_2 INTENSITY_3 INTENSITY_4 INTENSITY_5
INTENSITY_6 INTENSITY_7 INTENSITY_8 INTENSITY_9 INTENSITY_10 INTENSITY_11 INTENSITY_12
INTENSITY_13 INTENSITY_14 INTENSITY_15 INTENSITY_16 INTENSITY_17 INTENSITY_18
INTENSITY_19 INTENSITY_20 INTENSITY_21 INTENSITY_22 INTENSITY_23 INTENSITY_24
INTENSITY_25
FORMAT %4d %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e
%10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e %10.4e

#Tau(Hz): 0.0000e+00 0.0000e+00 1.0000e+01 2.0000e+01 3.0000e+01 4.0000e+01 5.0000e+01 6.0000e+01
7.0000e+01 8.0000e+01 9.0000e+01 1.0000e+02 1.2000e+02 1.4000e+02 1.6000e+02 2.0000e+02 2.4000e+02
2.8000e+02 3.6000e+02 4.2000e+02 5.0000e+02 6.0000e+02 7.0000e+02 8.0000e+02 9.0000e+02 1.0000e+03
# Key I0 I(1) I(2) I(3) I(4) I(5) I(6) I(7) I(8) I(9) I(10) I(11) I(12)
I(13) I(14) I(15) I(16) I(17) I(18) I(19) I(20) I(21) I(22) I(23) I(24) I(25)
 2 6.2284e+06 6.2284e+06 2.9604e+06 3.0765e+06 3.1479e+06 3.2925e+06 3.3360e+06 3.3950e+06 3.4216e+06
3.5487e+06 3.4628e+06 3.5930e+06 3.5867e+06 3.6721e+06 3.7080e+06 3.7893e+06 3.7995e+06 3.8886e+06
4.0248e+06 4.0006e+06 3.9613e+06 4.0772e+06 4.0269e+06 4.0476e+06 3.9671e+06 4.0461e+06
 3 1.3296e+07 1.3296e+07 7.0639e+06 7.1776e+06 7.1983e+06 7.2802e+06 7.3119e+06 7.4520e+06 7.3689e+06
7.4535e+06 7.4673e+06 7.5665e+06 7.6836e+06 7.7435e+06 7.7180e+06 7.9347e+06 7.9644e+06 8.0888e+06
8.1701e+06 8.1633e+06 8.2740e+06 8.3657e+06 8.3832e+06 8.3484e+06 8.3412e+06 8.4147e+06
 4 1.0207e+07 1.0207e+07 4.7410e+06 4.7475e+06 4.7177e+06 4.8062e+06 4.7626e+06 4.7482e+06 4.8615e+06
4.7874e+06 4.8743e+06 4.9218e+06 5.0399e+06 4.9891e+06 5.0268e+06 5.1012e+06 5.1770e+06 5.2190e+06
5.3743e+06 5.3726e+06 5.5419e+06 5.5647e+06 5.6520e+06 5.7722e+06 5.7673e+06 5.8209e+06
 5 2.1007e+06 2.1007e+06 1.0179e+05 1.5425e+05 1.3049e+05 9.8596e+04 1.5808e+05 1.6512e+05 5.0790e+04
1.6483e+05 7.5394e+04 1.8576e+05 8.0502e+04 1.5084e+05 2.1267e+05 2.6123e+05 2.3994e+05 3.3869e+05
3.3328e+05 3.0310e+05 5.4029e+05 4.6458e+05 6.3274e+05 6.7379e+05 6.9159e+05 8.1380e+05

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