FELIX 2002

Tutorials

MARCH 2002

*All updated documentation (**User Guide**, **Tutorials**, and **FELIX Command Language Reference**) for the latest release of FELIX is available at the Accelrys website documentation library:*

http://www.accelrys.com/doc/life/index.html

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> *FELIX 2002 User Guide*, March 2002. San Diego: Accelrys Inc., 2000.

Contents

How To Use This Book

The FELIX *Tutorials* are a set of step-by-step procedures that illustrate typical examples of how to use FELIX 2002 to process raw NMR data.

To access the tutorials, please use one of the following:

- Accelrys website (http://www.accelrys.com/doc/)[1]
- FELIX installation CD[2]

Any updates or corrections to the text files will be posted to the web site, making the text at www.accelrys.com the most current.

> **Note:** If you are prompted for a username and password at the Accelrys documentation website, use the following:
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An index and table of contents are provided. You can also use the Accelrys Site Search at:

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Select **All Documentation** in the **Search Area** list. Enter term(s) in the **Search** field, then click **Search**.

While viewing the document online, you can use the searching capabilities of your browser to locate information.

You can print individual chapters of these documents via your browser's printing capability.

[1]Contains only the text files; no tutorial data files are available on the Web.
[2]Contains both the text files and the data files used in the tutorials.

For information about the FELIX command language or to access the user guide, please see the online *FCL Command Language Reference* and FELIX *User Guide* books.

Who should use this documentation

This documentation is intended for FELIX users who want to become familiar with the program by working through tutorial lessons.

Topics covered

The tutorial lessons illustrate a wide range of topics, including:

- 1D, 2D, and 3D data processing.
- Analyzing relaxation data.
- Homonuclear and heteronuclear assignment.
- Using the Autoscreen module.
- Using the database.

Tutorial prerequisites

Before working with the FELIX tutorials, make sure you are familiar with the following:

- Basic operations of the Windows operating system.
- Use of the mouse on your workstation.
- The FELIX menu interface.

Workstation requirements

Before you begin, be certain that you have the following available on your workstation:

- An installed and licensed copy of FELIX 2002.
- A directory in which you can create subdirectories and files.

Setting up tutorial files

Before starting the tutorials, create a location on your hard drive to store tutorial files.

Create a folder on your hard drive to store tutorial files. For example:

```
> C:\Felix_Practice
```

Caution: Do not put the folder in the same path as the FELIX program files.

By default, FELIX 2002 program (executable) and tutorial files are installed in `C:\Program Files\Accelrys\`**Felix 2002**

Locate the tutorial files. They are located in a folder named `tutorials` in the same path as the FELIX program files.

To find these files, you can:

- Navigate through the directory structure using the Windows Explorer from either the **My Computer** icon or the **Network Neighborhood** icon on your Windows desktop.
- Right-click the **Felix** icon on the Windows desktop. Click **Properties**. On the **Shortcut** tab of the popup, note the location of FELIX in the Target window. For example:

  ```
  C:\Program Files\Accelrys\Felix 2002\felix-win.exe
  ```

  In this example, the `tutorials` folder are in the following path:

  ```
  C:\Program Files\Accelrys\Felix 2002\tutorial
  ```

Copy all the folders contained within the `tutorial` folder to the **Felix_Practice** folder. This uses about 270 MB of disk space. If you don't have enough disk space you may copy only some folders for selected tutorials.

Related books

You can find additional information about FELIX 2002, general molecular modeling, structure determination, and NMR data analysis in several other online books:

- *FCL Command Language Reference*—provides information for advanced FELIX users about using FCL and the command mode of FELIX.
- *Felix User Guide*—Contains general information about FELIX.
- *NMRchitect*—Describes the theory of NMR data analysis and how to use the NMRchitect software to analyze NMR data in the Insight II environment.
- *Insight II*—Describes the Insight II general molecular modeling program environment.
- *System Guide*—Provides step-by-step instructions for installing and administering Insight II products in your operating environment.

Typographical conventions

Unless otherwise noted in the text, this book uses the typographical conventions described below:

- Names of pulldowns, commands, and other items in the Felix interface are presented in **bold** type. For example:

  Select the **File** pulldown.

- FELIX command and file samples are presented in a `courier` font. If the example indicates something you must type, it is given in **`bold courier`** font. For example, type the FELIX command shown below into the command window when you want to open a file as writable:

  `mat yuin.mat w`

- In referring to the menu items that are used when running FELIX through its menu interface, this guide uses the format **Pulldown/Command**, since you use the mouse to select the pulldown first, before the command name appears. Where there is more than one cascading pulldown to access before the command name appears, the pulldowns are simply given in the order that you select them.

1 1D Processing

About the lessons

In *Lesson 1: Introduction to 1D processing*, you learn the basics of loading and processing 1D data files.

Topics:

- Loading 1D data files.
- Apodizing data.
- Fourier transforming data.
- Phasing.
- Baseline correction.
- Integration.

In *Lesson 2: Advanced 1D processing*, you learn some of the many options available for processing 1D data.

Topics:

- Working with multiple frames.
- Options for baseline correction.
- Working with buffers.
- Referencing.
- Using the cursor to measure J-coupling and chemical shift values.
- Peak picking options.
- Annotation.

Lesson 1: Introduction to 1D processing

This lesson introduces the FELIX novice to some of the commands FELIX uses to process a one-dimensional data set.

In this tutorial you will process an 1D NMR data set. Once you're comfortable with the procedures, this task takes approximately ten minutes to complete.

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*.

The files for this lesson are located in the **1D\Lesson1** folder.

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002**/**Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

FELIX displays an OPEN DATABASE FILE dialog box. Navigate to your working directory. If you used the default suggestion, this will be:

C:\Felix_Practice\1D\Lesson1

Enter a new filename (e.g., **test**) for a new empty database. When you enter the name of a brand new database file, FELIX will create the file.

By default, FELIX assigns the folder where you open the database file as the current working folder, although you can always change the working folder by selecting ***Preference/Directory****.*

Select the **File/Open** command. Set the **File Type** parameter to **Other Data [Bruker, Varian...]**. Next, navigate to the **tutorial\1D\Lesson1** folder. From the contents of this folder, select the **fid** file. Click **OK** to confirm the selection. FELIX opens the file in the window.

This loads the data. The graphics frame displays a 32768 point FID, which we can now process.

Routine 1D data processing often consists of removing **DC offset***, multiplying the FID by an exponential window function, transforming the results into the frequency domain, and phasing the spectrum to obtain pure absorption peaks. Subsequently, baseline roll is removed and the spectrum is integrated. We will follow these steps here.*

3. Removing DC offset

Select the **Process/DC Offset** command and choose **Oversample DBC** as Type. Click **OK** to perform the DC offset removal.

FELIX calculates a value for the baseline from the last 20% of the data points and subtracts that value from each data point.

4. Applying Window Function

Select the Process / Window Function command to obtain a control panel that prompts for a window function. Enter **Exponential** as the **Window function** and click **OK.** In the following control panel, enter 0.2 for **Line Broadening** and Click **OK** to multiply the data by an exponential window function.

The display is updated to show the results.

5. Fourier-transforming the data

Now, transform the data from the time domain to the frequency domain using the oversampled transform (these data were collected as a Bruker oversampled spectrum).

Select the **Process/Transform** command. FELIX determines that the transform type should be **Oversampled FFT**. Click **OK**.

The result is a spectrum in the graphics frame. Notice that the spectrum requires some phase correction. This is most easily applied by first using the phase parameter values from the procs file.

Select the **Process/Phase Correction** command. Set the **Method** to **Parameter** and leave the **Zero** and **First Order** parameters at their current values (136.912 and 14.55447, respectively). Click **OK**.

This should produce an almost perfectly phased spectrum. If you need to adjust the phasing, you can use the real-time phase interface.

To activate the real-time phase correction interface, select the **Process/Phase Correction** command and select the **Real-Time** option. Since this is a modeless dialog, you can still activate the main menu and toolbar icons to adjust the display of the spectrum while the phasing dialog is on. Use the REAL TIME PHASING dialog to adjust the **pivot** point position, the zero-order phase correction (**Phase0**), and the first-order phase correction (**Phase1**).

*Change pivot point by clicking the **Pivot** button and then click the desired spectral point. Change the phase values by sliding either of the sliders left or right. These changes are visible in the display as you make them.*

Repeat this with each correction until you are satisfied with the spectrum, then click **OK** to complete the phase correction.

This removes the real-time phase interface and makes the phased spectrum appear in the graphics frame.

6. Performing the baseline correction

Select the **Process/Baseline Correction** command. Toggle the Baseline Point to **on**. Leave the parameter at **Auto Pick Points** as is. Click **OK**. In the next control panel, leave the **Interval Size** at 128 and Maximum Deviation at 5. Click **OK**.

After FELIX selects the baseline points, it marks their locations with red tics at the bottom of the display frame.

In the BASELINE CORRECTION dilaog, **toggle the Baseline Correction** to **on a**nd select the **Polynomial** option. When the dilaog appears that prompts for the **Polynomial order**, click **OK** to select the default of **5** (fifth-order polynomial).

FELIX applies the polynomial function to the spectrum.

In the BASELINE CORRECTION dialog, click **Done** to close it.

7. Integrating and displaying the data

The last step is to integrate the areas of the peaks and display the integrals as a cumulative sum.

Select the **View/Draw Integrals** command.

FELIX calculates the integral of the peaks and displays the integral on the spectrum.

Note: This integrates the whole spectrum. For more sophisticated integration, you can use the **Measure/Integral** or **Volume** command.

8. Exiting FELIX

To exit FELIX, select **File/Exit** to begin the shutdown sequence. You either leave the **Save Current Session** and **Save Current Database** parameters on; or, toggle them to off. Then click **OK**.

This shuts down FELIX. If you choose to save the current session and save the current database, all of the following are saved: the current session and the baseline points or integrals in the database file you selected when the program started.

After this brief tour, you should be familiar with the basic 1D processing features of FELIX and how to navigate through the menu system.

Lesson 2: Advanced 1D processing

In this tutorial you read in a file of raw NMR data, process it to obtain a spectrum of resonances (peaks), and change the spectrum's display and annotations. This takes approximately 20 minutes to complete once you are comfortable with the procedures.

1. Setting up

If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*.

Locate the files listed below in the **1D\Lesson2** folder.

2. Starting FELIX

Start FELIX by double clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs** / **Accelrys** / **Felix 2002** / **Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

3. Selecting a database

The database is a binary file where FELIX stores data like segments, baseline points, peaks, volumes, etc. The **Edit/Table** *command gives you access to all data stored in the database. The* **File/Export/Table**

*and **File/Import/Table** commands allow you to read and write the data as ASCII files.*

FELIX displays an OPEN DATABASE FILE dialog box. Here you will create a database file.

Navigate to your working folder. If you used the default suggestion, this will be:

C:\Felix_Practice\1D\Lesson2

Then enter a new filename, such as **test2**. Click **OK** to complete the action.

Where you create or open the .dba file will determine your initial working directory. You can always change the working folder by selecting **Preference/Directory**.

4. Reading the data file

Now you proceed to manipulate files and workspaces.

Select the **File/Open** command. Set the following parameter values in the dialog box:

Filename	**sample.dat**
File Type	**Felix New Data**
Dimension	**1D**

Click **OK**

An FID (free induction decay; the raw NMR signal) appears in the frame. This is a Bruker file and must be processed accordingly.

The following steps will show you how to open and arrange multiple spectral windows (frames) in FELIX. You can ignore them and go directly to Step 6 *if you like.*

5. Creating multiple frames

Select the **Window/New Layout** command. Select **4 Small Square Frames**.

FELIX close the original window and displays four small windows, the last one being the active one.

Now select the **View/Plot** command.

FELIX plots the FID in frame 4 (the active window).

Activate Frame 1 by clicking its header. Select the **File/Open** command. Enter **sample2.dat** as the **Filename** and click **OK**.

FELIX displays a different FID in the active frame.

6. Creating additional frames

Create a new frame by selecting **Window/Add New**.

FELIX displays a fifth frame. All frames are automatically arranged.

Note: By default, FELIX automatically rearranges all spectral and table frames whenever a new frame is displayed. To turn off this feature, select **Preference/Frame Layout** and set **Option** to **None**.

To close Frame 5, click the **Close Window** button [**X**] in the upper right corner of the frame.

7. Returning to a single frame and reading the FID

Select the **Window/New Layout** command and choose the **1 Frame** option. This returns the screen to a single frame. Select **File/Open** to read in the **sample.dat** file as described in Step 4 above.

The above steps demonstrated how to open and arrange single or multiple spectral windows within FELIX.

8. Processing the data to create a spectrum

Select the **Process** / **DC Offset** command. Set the following parameter values in the control panel that appears:

Type	**BC**
Baseline Correct Fraction	**0.2**

Click **OK**.

Now select the Process / Zero Fill command and set these values:

Zerofill To	**16384**

Click **OK**.

Baseline correction corrects for the DC offset in a spectrometer. Zero filling adds zeros to the end of the FID. The default is twice as many points as the FID. The size of the FID is increased to this number of points.

Warning: If you execute this, beware that you have increased the size of your FID and resultant spectra. If you pop this to a buffer and then read in an 8K file, the buffer is also cut in half, and you lose half of your zero-filled spectra or FID!

9. Applying a window function

Select the **Process/Window Function** command. In the control panel, choose **Exponential** and click OK.

In the subsequent control panel set **Method** to **Real-Time**.

FELIX displays a new modeless dilaog that allows you to adjust the windowing function interactively. The mathematical function is drawn over the FID in red. Use the slider to change the parameters for the displayed window function and the effect on the FID.

Use the slider to set the **Line Broadening** parameter to a value of approximately 1.1.

Click **Keep**.

The FID is now multiplied with the window function and you see the results.

10. Transforming the data

Select the **Process/Transform** command. Select the **Bruker FFT** option.

Important: Data collected from some Bruker instruments is stored differently than data from other instruments. Hence, a special transform is needed

The Fourier transform produces the NMR spectrum.

Note: Newer versions of Bruker instruments can store data in a "normal" way. This is referred as "qsim" in the Bruker nomenclature. If a spectrum was recorded using this "qsim" parameter you must use the **Real FFT** command in FELIX

11. Phasing the spectrum

Select the **Process/Phase Correction** command. In the control panel set the **Method** to **Real-Time**. Click **OK**.

This is an interactive phasing mode. FELIX displays a modeless dialog box with sliders and buttons. You can activate the main menu items or the toolbar icons to change the spectral display.

Set the pivot for the spectrum by clicking the **Pivot** button under in the dialog and then clicking the cursor where you want the pivot point to be. Drag the upper slider until the peaks around the pivot point are properly phased.

12. Phasing an expanded region

Click the **Zoom** icon in the toolbar. Click-press-drag so that a rubber-band box covers the spectral region you want to zoom into. Release the button, and the selected region fills the window. Continue adjusting the **Phase0** and **Phase1** sliders to phase the spectrum. If necessary, click the **Coarse** or **Fine** button to increase or decrease the adjusting range, respectively.

Click the **Full plot** icon in the toolbar to view the entire spectrum. Click the **Increase threshold** icon to zoom in the y axis if necessary. When satisfied with the phasing, click the **OK** button.

13. Baseline-correcting the spectrum to eliminate the curved baseline

Select the **Process/Baseline Correction command.** Toggle the **Baseline Point** to **on** and choose the **Auto Pick Points** option. Click **OK**.

Click **OK** in the next dialog box.

This generates a set of baseline points displayed as small red lines under the spectrum to be used in the correction.

14. Adding user-defined base points

In the same dialog, select the **Pick Points Via Cursor** option. Click **OK**.

Next, move the vertical cursor to a point on the baseline that is not already selected with a red line, and click. This adds that point to the baseline. Repeat this process until you have defined all the desired baseline points. To quit the vertical cursor, press the <Esc> key.

15. Viewing the base points

Click the **Done** button to close the BASELINE CORRECTION dialog. Select the **Edit/Table** command. Double-click the bas directory and then double-click the **baseline** table.

FELIX displays a new window with a spreadsheet. You may scroll through the selected baseline points.

Note: When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

16. Applying the baseline correction

Close the table window by selecting **File/Close** from its own menu.

Select the **Process/Baseline Correction** command in the main menu. In the control panel, check **Baseline Correction** and select **Polynomial**. Click **OK** to apply the correction to the selected basepoints.

When the BASELINE CORRECTION dialog box displays again, click **Done** to close it.

Note: For finer adjustment of the polynomial used for baseline correction you may use the real-time **Polynomial** command. Here you may change the polynomial's parameters using the mouse and dial boxes. The display's appearance reflects the order of polynomial. You can change the polynomial parameters, up to the order of nine.

17. Using real-time sliders to compare two different spectra

Select the **Tools/Buffers/Store Work to Buffer** command. Set the **Buffer Number** to **1** and click **OK**.

This saves the data in the workspace to the first buffer. The data in the buffers are not displayed by default. The following step will force Felix to display the first buffer.

Select the **Preference/Plot Parameters** command. In the dialog box, set **Stack Depth** to **1**. Set **Color Scheme** to **Define**. Set **Color Cycle** to **2**. Click **OK**.

Two identical 1D spectra are displayed in different colors. The upper is the data in the first buffer and the lower is those in the workspace

Generally, when you open a data file or do data processing, the original data in the workspace gets overwritten. If you want to keep the original data, you must save them in a buffer before opening a new file. By default, you have up to five buffers with the same size as the workspace. You can change the number and size of the buffers using the Preference/ Memory command.

Click the **Plot Parameters** icon and set **Stack Depth** to **0**. This forces that only the data in the workspace is displayed.

Read the 1D file **sample.dat** into the workspace as in Step 4. Apply zero filling and a different window function, e.g., **Sinebell^2**, using **16384 and 90.0** for parameters.

Transform the FID (**Bruker FFT**) and use the **Process/Phase Correction** command with the **Parameter** option to apply the phase correction.

Finally, select **Process/Baseline Correction**, check **Baseline Correction** and choose **Polynomial**. Click **OK**. When the dialog box comes up again, click **Done** to close it.

Since the symbols (phase0 and phase1) and database entity (bas:baseline) that save the phasing parameters have not been overwritten, the same parameters and base points are applied to both spectra.

Click the **Plot Parameter**s icon and set **Stack Depth** to **1**. This forces that the two data sets in the workspace and the first buffer are displayed.

Select the **Preference/1D Scale** command. Set **Scaling** to **Absolute**. To put the red spectrum on top of the white one, move the **Overlap** slider and set it to 1.0. The difference between the two should be noticeable.

Click **OK** to leave the real-time sliders.

18. Getting the difference between two spectra

Select the **Tools/Buffers Subtract from Buffer** command. Set the **Buffer Number** to **1**, then click **OK**.

This subtracts the data in buffer1 by those saved in the workspace. The difference spectrum is displayed as buffer 1. The data in the workspace remain unchanged.

Select the **Preference/Plot Parameters** command. In the dialog box, set the **Stack Depth** to **0**, then click **OK**.

This displays only the spectrum in the workspace.

19. Viewing an expanded region

Click **Zoom**. Click and hold the left mouse button and drag the mouse so that the rubber box covers the region around the water resonance (~7000-9000 points). Release the left mouse button.

20. Setting the reference on water peak

Select the **Preference/Reference** command. Click the **Cursor** button, and the cursor becomes a vertical line. Now click the water resonance (at ~8200 points). FELIX displays the control panel again. From the **Axis Type** popup select the **Ppm** option. Set **Reference PPM** to **4.76** and click **OK**.

Felix redisplays the spectrum with new ppm units.

Click the **Full Plot** icon in the toolbar. Or, you may press <Ctrl>+f on the keyboard.

21. Getting chemical shift information

Click the **Zoom** icon and zoom in to the downfield region of the spectrum at approximately 8.3-7.1 ppm.

Select the **Measure/Cursor Position** command (or you can click the **Cursor Value** icon in the toolbar). The cursor becomes a vertical half-crosshair. Move the cursor over the spectrum. Information on the chemical shift and peak height appears on the status bar. To exit, press <Esc>.

22. Getting J-coupling information

Select the **Measure/Distance/Separation** command. Click the tops of two neighboring peaks, such as the set at 7.71 and 7.69 ppm. The separation value, ~ 7 Hz in this example, appears on the status bar.

To exit, press <Esc>.

23. Identifying peaks

Select the **Preference/Pick Parameters** command. Click the **Cursor** button. When the cursor becomes a large horizontal line, position it to give a threshold below the lowest peak and click the mouse button. In the control panel, acknowledge the threshold by clicking **OK**.

The threshold is the "lowest" intensity defined as a peak and is used to avoid selecting noise as peaks.

Select the **Peaks/Pick All** command. Click **OK**.

This labels all peaks, and displays a peak table.

Now select the **Peaks/Remove Region** command. Again drag to select a region.

FELIX removes the labels from the deselected peaks.

Select the **Peaks/Pick One** command (or right-click the spectrum and then select the **Pick One Peak** item from the context menu); click the tops of a few of the deselected peaks; then press <Esc> to display the values.

Select the **Peaks/Remove All** command. Click **Delete**.

24. Annotating the spectrum

Select the **Edit/Annotation** command (or click the **Annotate** icon from the toolbar). Enter a name **ann** for the annotation file in the resulting control panel and click **OK**.

FELIX displays a modeless ANNOTATIONS dialog.

Click the **Roman Text** button. Move the large crosshair to the desired starting point for text inside the frame and click the mouse button. Type your text (such as **peak 1**) in the **Text** box and click **OK**.

Click the **Arrow** button. Move the crosshair underneath the text, and drag it towards the top of a peak.

Click the **Parameters** button.

Notice that many options can be adjusted in the new control panel.

Click **Cancel** to exit the control panel, then click **OK** to leave the **Annotations** control panel.

25. Exiting FELIX

To exit FELIX, select the **File/Exit** command.

2 2D Processing and Analysis

About the lessons

In *Lesson 1: 2D processing, display, and analysis*, you will learn to process, display, and analyze 2D matrix files.

Topics

- Reading and processing 1D data blocks from a 2D data set.
- Determining processing parameters for the D1 dimension.
- Processing the D1 dimension.
- Examining individual 1D vectors.
- Processing the D2 dimension.
- Opening and displaying matrix files.
- Setting display parameters.
- Adjusting plot parameters.
- Picking peaks.
- Using lists.
- Assigning peaks.
- Tiling cross peaks.

In *Lesson 2: Analyzing Relaxation Data*, you will learn to perform a relaxation analysis.

Topics

- Accessing the **Relaxation** menu.
- Measuring peak heights in an R_1 series of spectra.
- Evaluating signal / noise ratios for peak heights.
- Viewing a time course.
- Fitting R_1 values to a time course.

- Evaluating R_2 data.
- Evaluating heteronuclear NOEs.
- Generating Modelfree input.

Lesson 1: 2D processing, display, and analysis

In this lesson you process a 2D matrix, display the spectrum using a variety of standard plotting methods, and assign the cross peaks. You start by processing a 2D matrix using the **EZ macros**.

This lesson takes approximately 60 minutes to complete once you are comfortable with the procedures.

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*.

The files for this lesson are located in the **2D\Lesson1** folder.

When FELIX processes data it expects to see the data with the same directory structure as existed on the spectrometer. So the whole 2D\Lesson1 folder must be copied to keep the directory structure.

2. Starting FELIX

Start FELIX by double clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002** / **Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

FELIX displays an OPEN DATABASE FILE dialog box. Navigate to your working directory (e.g., **2D \Lesson1**), and type a new filename, such as **test**. Click **OK**.

Where you create or open the .dba file will determine your working directory. To change the working folder, select ***Preference/Directory****.*

3. Reading in the first FID

Select the **File/Open** menu item. Set the **File Type** parameter to **Other Data (Bruker, Varian...)**. Locate and open the **1** folder (the experiment directory) to open it. Locate and click on the **ser** file to select it. Click **OK** to open the file.

FELIX displays the first FID of a 2D HSQC spectrum collected on a Bruker spectrometer.

Instead of going directly to 2D processing, first we will apply a few 1D processing functions on the displayed FID in order to get some spectral parameters.

4. Apodizing the FID

Select the **Process/Window Function** command. Select **Sinebell^2** as the apodization function. In the next control panel, leave the default parameters (512 and 90.0) and select the **Real-Time** option for **Method**.

*FELIX displays the FID along with the apodization function, colored red. You may experiment with different settings of the **wsize** and **wshift** parameters, and watch their effect on the FID and the processed spectrum.*

Set the **Window Size** parameter to **512** and the **Phase Shift** parameter to **90**. Then click **Keep**.

FELIX displays the apodized FID on the screen.

5. Transforming the FID

Select the **Process/Transform** command. The default is **Complex FFT**. Click **OK**.

6. Phase-correcting the spectrum

Select the **Process/Phase Correction** command. In the control panel, select the **Real-Time** option for **Method** and click **OK**.

Click the **Pivot** button in the REAL-TIME PHASING dialog, and click a peak in spectrum which you would like to use for zero-order phasing. Using the first slider to adjust the **Phase0** parameter to phase this peak, then adjust the **Phase1** parameter as necessary.

Click **OK** when you are finished.

Since this is a modeless dialog, you can activate the main commands or the toolbar icons to change the spectral display when you are phasing the spectrum.

Now that you have a rough idea of the apodization and phase correction parameters, you can proceed with processing the D1(t2) dimension of the 2D data set.

Caution: Record the apodization and phase correction parameters on paper. The FELIX symbols that store these parameter may get overwritten in the following steps.

*You will now open the **ser** file again to process a 2D spectrum.*

7. Processing the D1 (t2) dimension of the 2D data set

Select the **Process/2D Data Processing** command. In the first control panel, select the previous ser file. In the next control panel, leave most of the header parameters at their default values (read from the spectrometer header files), except for these:

D1 Data Size	**512**
D1 Spectrometer Frequency	**600.1408**
D1 Sweep Width	**7002.801**
D2 Data Size	**32**
D2 Spectrometer Frequency	**60.8**
D2 Sweep Width	**3000**
Source	**Bruker**

Click **OK**.

In the next control panel set the following parameter values:

Data Type	**Complex**
Acquisition in D2	**Echo/Anti-Echo**

Click **OK**.

In the next control panel, set the following parameter values:

Dimension To Process	**D1 FT**
Output Matrix Filename	**bruker.mat**
Dimension 1 Size	**1024**
Dimension 2 Size	**128**
Correct DC-offset	**off**
Correct 1st-point	**none**
Solvent Suppression	**none**
Window Function	**Sinebell^2**
FT Type	**Complex**
Phasing Mode	**Use Parameters**
Phase0	**-84.0**
Phase1	**0.0**
Baseline Correction	**none**
Reverse Vector	**off**
Extract Half Spectrum	**Left Half**
Output Level	**Verbose**
Display Matrix	**on**

Click **OK.**

The processed data will be saved in a FELIX matrix file named *bruker.mat* in this example. This file will be located in the folder designated for FELIX matrices. To change that folder, interactively, press the **...** button next to **Output Matrix Filename** and navigate to the folder you want.

When the **Sinebell Parameters** control panel appears, enter these values:

Data Size (Points)	**512**
Phase Shift (Degrees)	**90.0**

Click **OK**.

Click **OK** again to start the D1 transform.

As the D1 transform proceeds, the progress is illustrated in the status bar. This step is often completed in less than one second.

After the first dimension is processed, FELIX shows the control panel for processing the second dimension.

8. Processing the D2 (t1) dimension

Set the following parameter values in the control panel:

Dimension To Process	**D2 FT**
Load Matrix in Memory	**off**
Processing Mode	**bundle**
Correct 1st-point	**none**
Solvent Suppression	**none**
Window Function	**Sinebell^2**
Linear Prediction	**on**
FT Type	**Complex**
Phasing Mode	**Use Parameters**
Phase0	**0.0**
Phase1	**0.0**
Baseline Correction	**none**
Reverse Vector	**off**
Output Level	**Verbose**
Display Matrix	**On**

Click **OK**.

In the **Linear Prediction** control panel, set these parameters:

First Point	**1**
Last Point	**32**
Start Point	**33**
End Point	**98**
Number of Coefficients	**8**
Method	**Forward-Backward**

Click **OK**.

In the **Sinebell Parameters** dialog box, set these parameters:

Data Size (Points)	**98**
Phase Shift (Degrees)	**90.0**

Click **OK**.

Click **OK** to start the D2 transform.

FELIX usually completes this step in a few seconds.

When processing completes,FELIX opens the matrix since you chose to display the matrix at completion. The contour threshold is calculated, and the matrix is then displayed.

9. Display the 2D matrix

FELIX calculates the plot levels. You can change them manually

Select the **Preference/Plot Parameters** command (or click the **Plot Parameters** icon in the toolbar). Set the following values:

Contour Threshold	**0.01**
Color Scheme	**Fire Ramp**

Leave the other values set at their defaults and click **OK**.

Select the **Intensity** or **Contour** options from the combo box in the toolbar to display the intensity map or contours of the spectrum, respectively.

Click the **Autoscale** icon in the toolbar to toggle between the proportional mode and fill-in mode.

Click the **Increase Threshold** and **Decrease Threshold** icons in the toolbar to raise or lower the threshold for spectral display.

While displaying the spectrum, the data are always read from the matrix, not from a graphics file, so that display parameters can be recalculated at any time. Hence, FELIX redraws the graph with each plot command.

At this point you could examine the D1 and D2 vectors in more detail to determine whether further baseline correction or phasing adjustments are necessary.

10. Referencing the matrix

Referencing of the matrix happens automatically, since the header parameters are read or adjusted during processing. You can further adjust the referencing, for example, by giving more descriptive names for the axis.

Select the **Preference/Reference** command. In the control panel, leave every parameter at its current value, except for these:

Axis Text D1	**D1_HN**
Axis Text D2	**D2_N15**

Click **OK**.

The 2D spectrum should now be redisplayed with the correct referencing for each axis.

11. Viewing an expanded-region contour plot

You can choose expanded regions with the cursor or by inputting numeric parameters:

Select the **View/Limits/Set Limits** command (or click the **Zoom** icon in the toolbar). Drag a box around the region that you want to expand.

When you release the mouse button, the region selected expands to fill the window. The plot is still in intensity mode, hence the contour levels are not shown.

Select the View / **Limits/Manual Limits** command.

In the control panel that appears, the parameters are filled in with the values of the current *plot.*

Set these parameter values to expand the region:

D1_HN lower	**7**
D1_HN upper	**9**
D2_N15 lower	**104**
D2_N15 upper	**141**
Limit Type	**ppm**

Click **OK**.

FELIX displays the new 2D region.

You can save these parameters and reuse them for other plots; for example, if you were analyzing a series of spectra collected with different mixing times and always wanted to observe identical regions.

Select the **View/Plottype/Contour** menu item to redraw the region as a contour plot.

FELIX now plots the 2D matrix in contour mode, with a color-coded intensity scale.

12. Changing the 2D drawing parameters

If no peaks are visible, try decreasing the contour level to cut lower into the spectrum. If the peaks are outlined but you do not see the circles shrinking to define the tops of the resonances, try increasing the level multiplier to increase the space between levels:

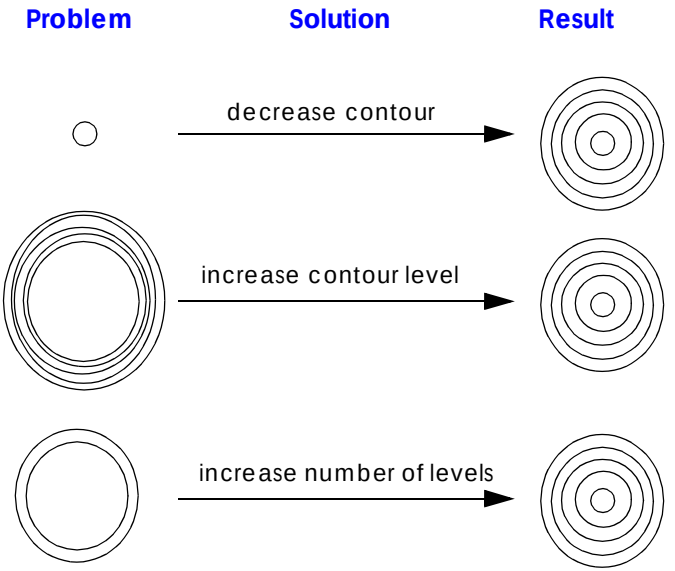

Figure 1

13. Showing the grid display

Select the **Preference/Plot Parameters** command. Click the **Axis** button. Set **Grid Spacing** to **3** and click **OK**.

FELIX displays three grid lines between each pair of major tick marks.

Select the **Preference/Plot Parameters** command. Click the **Axis** button and then set **Grid Spacing** to **0** and click **OK**.

FELIX displays no grid lines.

14. Returning to the full spectrum

Click the **Full Plot** icon in the toolbar or press <Ctrl>+f to return to the full spectrum.

Note: If you select **Full Limits** while in contour mode, the spectrum may take a long time to redraw for a big matrix. To stop plotting, press <Esc> on your keyboard. Then, redraw the spectrum in intensity map by selecting the **Intensity** option from the **Plot Type** combo box in the toolbar.

15. Picking peaks

First, set the peak-picking parameters. Select the **Peaks/Pick Region** command. Leave the parameters at their default values, but set the **Pick Region Mode** to **Define by Cursor**. Click **OK**.

Use the cursor to drag out a box that includes the entire set of desired peaks.

FELIX displays red boxes around all cross peaks meeting the criteria defined in the control panel.

A new window containing the peak table is displayed to the left of the spectral window. Note that, by default, whenever a new window (table or spectral) is open, FELIX automatically re-arranges the layout of the windows. You can turn off this feature by selecting ***Preference/Frame Layout*** *from the main menu and set* ***Action*** *to* ***None****. You can also do the automatic re-arrangement at anytime by selecting* ***Window/Auto Arrange****.*

Note: When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

16. Deleting peaks and replacing them

Activate the spectral window by clicking on it. Select the **Peaks/Remove One** command (or right click inside the spectral window and select **Remove One Peak** in the context menu). The cursor becomes a +. Click one or two red boxes to remove them, then click in the empty space to the left of the frames to return to a normal cursor.

Note: FELIX updates the spreadsheet when you remove peaks.

Select the **Peaks/Remove Region** command. Drag a box around a few peaks to be deleted.

Select the **Peaks/Pick Region** command and repeat the dragging to add another region of peaks.

Select the **Peaks/Pick One** command (or right click inside the spectral window and select **Pick One Peak** in the context menu) and click to add individual peaks. Click in the empty space to the left of the frames to return to a normal cursor.

Click the **Zoom** icon in the toolbar and zoom into a few peaks. Select the **Peaks/Edit** command (or right lick side the spectral window and select **Edit One Peak** in the context menu) to manually adjust the box defining a peak.

Click in the center of a red box of a picked peak. The box becomes green, indicating that it is selected for editing. Click the center of the box and drag to move the entire box or click near a corner of the box and drag to resize it.

To leave editing mode, press the <Esc> key while the spectral window is activated.

Select the **View/Plot** command to redraw the window (and clean up any broken lines or other details).

17. Assigning the cross peaks

Activate the spectral window and click the **Plot** icon to clean up the display.

Select the **Peaks/Name One Peak** command and click one of the red boxes.

Set the following parameters to assign the resonance:

Item number	**148** (or whatever it is listed as)
D1 peak name	**h1**
D2 peak name	**null**

Click **OK**, then press the <Esc> key to return to a normal cursor.

You do not need to assign the peak in both dimensions. You can label the cross peaks in one dimension at a time, as the assignment is made. This is usually how assignments are observed.

If you want to use the restraints list directly in the Insight II or Discover program, you have to use the Insight II proton names as the peak names. At the moment there is no check of atom names, so you may enter anything you want.

18. Displaying assignments

Select the **Peaks/Find** command, select **Find Peak By Name** and set the **Action** to **Color**. Set the **Peak Name D1** to **h1** and **Peak Name D2** to *****. Select **Yellow** for **Color**. Click **OK**.

*FELIX searches the assignment list, and colors the boxes surrounding all peaks with a label of **h1** in D1 dimension in yellow. FELIX reports the coordinates of the peak on the status bar.*

19. Calculating volumes

To calculate the volumes of the picked peaks, select the **Measure/Integral/Volume** command. In the control panel, select the **Measure All Volume** option for **Action**. In the next control panel, leave the **Peak** and **Volume** set at their defaults. Set **Volume Slot Number** to **1** and **Mixing Time** to **0.1**. Click **OK**.

FELIX displays the BUILDING NEW VOLUME ENTITY control panel; set **Total Mixing Time Slots** to **6** and click **OK**.

You can now view the volume data with the **Edit/Table** command (selecting the **vol:volumes** entity) or write the volume data to a file with the **File/Export/Table** command from the main menu.

To calculate restraints from these volumes based on the two-spin approximation, you must open or create a scalar entity for the database, define a scalar pair, create the restraints (strong–medium–weak, or any other listed choice), and write the restraints file. The appropriate commands are in the ***Measure*** *menu.*

20. Quit FELIX

To quit FELIX, select the **File/Exit** command.

Lesson 2: Analyzing Relaxation Data

In this lesson you learn to perform a relaxation analysis based on analyzing heteronuclear relaxation data. It is assumed that R_1, R_2, and heteronuclear NOE were measured as a series of 2D HSQC (or equivalent) spectra. The data used are parts of the relevant spectra acquired for apocalbindin D_{9k} (Akke et al. 1993).

1. Setting up for the lesson

See “Setting up tutorial files” in the preface, *How To Use This Book* as needed.

Locate the files listed below in the **2D \Lesson2** folder. Each is required for this lesson.

t1_1.mat	**t1_7.mat**	**t2_6.mat**
t1_1b.mat	**t2_1.mat**	**t2_7.mat**
t1_2.mat	**t2_1b.mat**	**noe1.mat**
t1_3.mat	**t2_2.mat**	**noe2.mat**
t1_4.mat	**t2_3.mat**	**noe3.mat**
t1_5.mat	**t2_4.mat**	**noe4.mat**
t1_6.mat	**t2_5.mat**	**relax.dba**

2. Start FELIX

Start FELIX by double clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002** / **Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, navigate to your working directory (e.g., **2D \Lesson2**), and select the **relax.dba** file from the list. Click **OK**.

The name and path of the database file appears on the title bar of FELIX main window.

Note: Most of the commands for relaxation analysis are in the pullright menu that appears when you select **Measure/ Relaxation**. In the following sections, we call this pullright menu the “**Relaxation** menu.”

3. Measure peak heights in the R_1 series of spectra

Select the **Measure/Relaxation/Measure Heights/ Volumes...** command. In the control panel, set these values:

Peak table	**xpk:peaks**
Volume Table	**vol:t1**
Number of Spectra	**7**
Measure	**Peak Heights**

Click **OK**.

In the next control panel, FELIX prompts for the first spectrum in the series and some parameters.

Select the **t1_1.mat** matrix and set these values:

Volume Slot:	**1**
Relaxation Delay: (s)	**0.03**

Click **OK**.

FELIX plots the spectrum, repositions the peaks to their exact centers. and calculates the peak heights.

When the spectrum selection control panel appears again, you need to specify file names and parameters for the remaining spectra in the same way as you did above.

Enter the following values (in this sequence):

File name	Slot	Relaxation Delay
t1_2.mat	**2**	**0.11**
t1_3.mat	**3**	**0.24**
t1_4.mat	**4**	**0.48**
t1_5.mat	**5**	**0.96**
t1_6.mat	**6**	**1.5**
t1_7.mat	**7**	**2.94**

Click **OK**.

To view the volume table, select the **Edit/Table…** command from the FELIX toolbar and double-click **vol**. Next click **t1** and click **OK**.

FELIX displays the relaxation delays in the last row of the table.

4. Evaluate the signal/noise ratio for the peak heights

Select the **Signal/Noise Ratio…** command in the **Relaxation** menu. In the control panel, enter:

Number of Spectra:	**1**
\Volume Entity:	**vol:t1**
Error Entity:	**vol:t1s**
Evaluate:	**Peak Heights**

Click **OK**.

In the next control panel, enter:

File name	**t1_1b.mat**
Relaxation delay:	**0.03**

Click **OK**.

FELIX calculates the peak heights in this duplicate spectrum, calculates the average height difference between this spectrum and its twin spectrum, and derives the uncertainty of the volume determination.

FELIX reports these values in the text-prompt window. If you have more than one duplicate time point in your relaxation series, the uncertainties for the other time points are interpolated or extrapolated. For a single duplicate measurement, the uncertainties are promoted to the other points.

To view the S/N table, select the **Edit/Table...** command from the FELIX toolbar and double-click **vol.** Next click **t1s** and click **OK**.

FELIX displays the uncertainties in the last row of the table.

5. View a time course

Select **View Timecourse via Cursor** in the **Relaxation** menu and then click a peak in the displayed spectrum.

FELIX plots a graph of the peak height vs. relaxation delay, including error bars. Due to the good S/N in the spectra, the error bars may not be immediately apparent.

To see details more clearly, maximize the frame, clicking the **Maximize** button in its upper-right corner.

Click the **Restore** button to return the graph to its original size.

To view a time course by entering a peak number, select **View Timecourse via Item** in the **Relaxation** menu and enter a number in the dialog box.

FELIX displays the corresponding time course or informs you that no such peak number exists and lets you try again.

To print the plot use the FELIX printing function (either click the **Print** icon or select the **File/Print…** command).

FELIX uses data from the currently active tables to display the time courses. If you want to view data from different tables, use the ***Preference/Table…*** *command to assign another relaxation table.*

6. Fit R_1 values to the time courses

Select the **Fit R1/R2/NOE** command in the **Relaxation** menu. In the control panel, select **Fit R1 Timecourse** and click **OK**.

In the second control panel enter:

Volume Table	**vol:t1**
S/N Table	**vol:t1s**

Click **OK**.

FELIX now fits the time course data to the exponential function:

$$y = a_0 + a_1 e^{a_2 x} \qquad \textbf{Eq. 1}$$

and derives the relaxation rate R_1 from the coefficient a_2 in the exponent. FELIX reports the relaxation rate R_1, its standard deviation, and the χ^2 value for each fit in the text window.

*FELIX stores the following in the table **rel:r1**: relaxation rate R_1, the raw coefficients for the offset a_0 and linear term a_1 in the function, and their standard deviations.*

In addition, FELIX stores the χ^2 value of the fit for each time course.

To view the rel:r1 table select the **Edit/Table…** command from the FELIX toolbar and double click **rel.** Next select **r1** and press **OK**.

*If you now view a time course, FELIX plots the fitted function in red, along with the experimental peak heights. This lets you visually judge the quality of the fit. You can print the plot by clicking the **Print** icon.*

7. Evaluate R_2 data

To evaluate R_2 data repeat Steps 3 through 6 with these values:

File name	Slot	Relaxation Delay
t2_1.mat	**1**	**0.004**
t2_2.mat	**2**	**0.032**
t2_3.mat	**3**	**0.092**
t2_4.mat	**4**	**0.180**
t2_5.mat	**5**	**0.300**
t2_6.mat	**6**	**0.702**
t2_7.mat	**7**	**1.300**
t2_1b.mat		**0.004**

*The **t2_1b.mat** is a duplicate spectrum. R_2 time courses are fitted to the simple exponential function:*

$$y = a_0 e^{a_1 x} \qquad \textbf{Eq. 2}$$

and the general exponential function:

$$y = a_0 + a_1 e^{a_2 x} \qquad \textbf{Eq. 3}$$

Whichever function yields the lower χ^2 value is used to derive the R_2 relaxation rate. FELIX reports the R_2 value, its standard deviation, and the χ^2 value in the text window and also tells you which function was used.

All the fitted values are stored in the table rel:r2, analogous to the R_1 data (see Step 6).

8. Evaluate heteronuclear NOEs

Select the **Fit R1/R2/NOE** command on the **Relaxation** menu. Select **Evaluate NOE** in the control panel and click **OK**.

In the next control panel enter:

Peak Table:	**xpk:peaks**
Volume Table:	**vol:noe**
NOE Table:	**rel:noe**
Measure:	**Peak Heights**

Click **OK**.

In the third control panel, select **noe2.mat** as the spectrum *with* 1H saturation and click **OK**.

In the fourth control panel, select **noe1.mat** as the spectrum *without* ^{1}H saturation and click **OK**.

FELIX plots the first spectrum, repositions peaks to their exact centers, and measures peak heights. Then it plots the second spectrum and measures peak height.

In the fifth control panel enter:

Peak Table:	**xpk:peaks**
Volume Table:	**vol:noes**
Measure:	**Peak Heights**

Click **OK**.

In the sixth control panel, select **noe4.mat** as the duplicate spectrum *with* ^{1}H saturation and click **OK**.

In the seventh control panel, select **noe3.mat** as the duplicate spectrum *without* ^{1}H saturation and click **OK**.

FELIX plots the first duplicate spectrum, repositions peaks to their exact centers, and measures peak heights. Then it plots the second spectrum and measures peak heights. Finally it reports the NOEs and their standard deviations to the text window and stores them in the table rel:noe.

To view the rel:noe table, select the **Edit/Table** command from the FELIX toolbar and double-click **rel.** Next select **noe** and click **OK**.

9. Generate Modelfree input

Once you have all R_1, R_2, and NOE values evaluated and stored in the database, you can generate an input file for the Modelfree program (A. G. Palmer, Columbia University, http://www.hhmi.columbia.edu/palmer/).

Select **Modelfree Input** from the **Relaxation** menu. Enter the following values in the control panel:

Nucleus:	**N15**
Spectrometer Frequency:	**500.13**
Number of Simulations:	**200**
Rot. correl. time (ns):	**4.25**
Step:	**0.2**
Modelfree file name:	**mfin.txt**
Tmest file name:	**tmest.txt**

Click **OK**.

Now you have initial input files for the tmest and Modelfree programs. For more information about working with Modelfree, please refer to its documentation and to the scientific literature.

10. Exit FELIX

Exit FELIX by selecting the **File/Exit** command

3 3D Data Processing

About the lesson

In this lesson you learn the basic processing steps involved in transforming 3D time-domain data to frequency-domain data. The lesson leads you through the processing and basic display a 2D slice for a 3D data set.

Topics

- Phase correction.
- Fourier transformation.
- 3D display manipulation.
- 2D slice manipulation.
- Peak picking of 3D data.

This lesson takes approximately 30 minutes to complete once you are comfortable with the procedures.

Lesson 1: 3D Data Processing and Display

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in “Setting up tutorial files” in the preface, *How To Use This Book*.

The files for this lesson are located in the **3D\Lesson1** folder.

*The sample data set is an HNCO experiment on ubiquitin, a 76-residue protein; the unprocessed data (the **fid** and **procpar** files) should be located in the **Felix_Practice\tutorial** directory before you start.*

2. Starting FELIX

Start FELIX by double clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

FELIX displays an OPEN DATABASE FILE dialog box. Here you will create a database file. Navigate to a working directory (e.g. **3D\Lesson1**)

Enter a new filename (e.g. **test**) for a new empty database.

3. Transforming the data

Select the **Process/3D Data Processing** menu item.

In the SELECT SPECTRUM FILE control panel, make sure the **Filter Type** is set to **All Files**, then select **fid** from the **Files** list and click **OK**.

The second control panel displays the header parameters. This is Varian States 3D data, which should have the following default parameters:

D1 Parameters

Data Size	**512**
Spectrometer Frequency	**599.945**
Sweep Width	**8000.001**

D2 Parameters

Data Size	**32**
Spectrometer Frequency	**150.869**
Sweep Width	**2431.88**

D3 Parameters

Data Size	**50**
Spectrometer Frequency	**60.797**
Sweep Width	**3000.075**

Environment

Pulse Program	**gCT_hn_co**
Solvent	**D2O**
Temperature	**303.0**
Data Source	**Varian**

Once you have verified that all the parameters are set correctly, click **OK**.

The third control panel now displays the acquisition parameters.

The **Acquisition Parameters** should be set like this:

Data Type	**Complex**
Acquisition Mode	**States or States-TPPI**
First Incremented	**d3, d2**
Quartet Order	**phase2, phase**

Once you have verified that all the parameters are set correctly, click **OK**.

In the fourth control panel, enter these parameter values:

Dimension To Process	**D1 FT**
Output Matrix Filename	**hnco.mat**
Dimension 1 Size	**512**
Dimension 2 Size	**64**
Dimension 3 Size	**128**
Correct DC-offset	**on**
Fraction	**0.5**
Correct 1-st point	**None**
Solvent Suppression	**None**
Window Function	**Sinebell**
FT Type	**Complex**
Phasing Mode	**Interactive**
FID to phase	**1**
Baseline correction	**None**
Reverse Vector	**off**
Extract Half Spectrum	**Left Half**
Output Level	**Verbose**

Click **OK**.

Note: The processed data will be saved in a FELIX matrix file named hnco.mat in this example. This file will be located in the folder designated for FELIX matrices. To view or change that folder, select **Preference/Directory** before 3D processing.

If you want to specify the folder interactively, you can click the **Ö** button next to **Output Matrix File** and navigate to the desired folder and specify a filename.

In the **Sinebell Parameters** control panel, verify that these parameters are set:

Method:	**Parameter**
Data Size:	**512**
Phase Shift:	**90.0**

Click **OK**.

FELIX displays the real-time phasing interface.

Adjust **phase0** (and **phase1**, if necessary). Click **OK** when you are done phasing.

Next FELIX builds the matrix.

When FELIX prompts you to initiate the transform, click **OK**.

When the processing of the D1 dimension has finished, FELIX should displays the message:

```
D1(t3) transform completed.

Elapsed time was 40 seconds
```

In this tutorial we continue the processing with the second dimension.

4. Transforming the D2 dimension

In the control panel, enter these parameter values:

Dimension To Process: D2 FT
Load Matrix in Memory:off
Processing Mode: Bundle
Correct 1st-point: None
Window function: Sinebell
Linear Prediction: off
FT Type: Complex
Phasing Mode: Use Parameters
Phase0: 0
Phase1: 0
Baseline Correction: None
Reverse Vector: off
Output Level: Verbose

Click **OK**.

In the next control panel, enter **Parameter, 32,** and **90** for the **Sinebell Window** parameters. Click **OK** to start the D2 transform.

In the output window, FELIX reports that there are 65536 D2 vectors to process. This is 512 x 128, or D1 x D3.

When FELIX has finished processing the D2 dimension you can continue with the third dimension.

In the next control panel enter these parameter values:

Dimension To Process:	**D3 FT**
Load Matrix in Memory:	**off**
Processing Mode:	**Bundle**
Correct 1st-point:	**None**
Window function:	**Sinebell**
Linear Prediction:	**off**
FT Type:	**Complex**
Phasing Mode:	**Use Parameters**
Phase0:	**0.0**
Phase1:	**0.0**
Baseline Correction:	**None**
Reverse Vector:	**off**
Output Level:	**Verbose**

Click **OK**.

In the control panel, enter **Parameter, 50**, and **90** for the **Sinebell Window** parameters. Click **OK** to start the D3 transform.

Now FELIX reports that there are 32768 D2 vectors to process. This is 512 x 64, or D1 x D2.

When FELIX has finished processing the D3 dimension, again open the matrix and display it.

5. Reading in the matrix

Select the **File/Open** menu item or click the **Open** icon.

File Type:	**Matrix**
Filename:	**hnco.mat**
Access:	**Read only**
Storage:	**Keep on Disk**

Click **OK**.

FELIX calculates the contour level automatically before displaying the spectrum. You can click the Increase Threshold or Decrease Threshold icon to adjust it.

Move the slider in the REAL-TIME PLANE dialog box to view other D1-D2 planes. You can also use the left or right arrow key to step through the planes when the slider is highlighted. Click **OK** to close it.

Click the **Orthogonal 1D Slice** icon, then click the cursor on a peak to view the D3 slice. Select the **Contour** option from the combo box in the toolbar to display the 3D spectrum again. You can repeat this step several times to check the D3 slices for more peaks. You should notice that D3 dimension is off phase.

6. Automatic phase correction in D3 dimension

Make sure the 3D spectrum is displayed. Select the **Contour** option from the combo box in the toolbar if necessary.

Select the **Process/Phase Correct Matrix** menu item. In the **Parameters** control panel, enter these parameter values:

Rephase:	**D3 vectors**
Phasing Mode:	**Automatic**

Click **OK**.

In the next control panel, check #1. Click the **Cursor** button. Drag the cursor so that the rubber-band box covers the upper field solvent peaks. After that, the control panel should display a range close to **From 451 to 512**. Click **OK**.

This excludes the solvent peaks from being considered while automatic phasing using the PAMPAS algorithm (Dzakula, 2000). It takes about half a minute to search all the D3 vectors for test peaks, calculate the phase errors, and then apply the phase correction to the whole matrix.

7. Picking the 3D peaks

Select the **Peaks/Pick All** menu item. In the Parameters control panel, enter these parameter values:

Peak Halfwidth

D1 Minimum:	**1.1**
D2 Minimum:	**0.7**
D3 Minimum:	**1.1**

Leave all other parameters at their current values and click **OK**.

When the peak picking finishes, a spreadsheet appears with the picked peaks.

8. Coloring peaks by slices

Select the **Preference/Peak Display** menu item. In the PEAK DISPLAY PREFERENCE control panel, select **Item #** as **Label Peaks**, change **Label Size** to **0.15** (inch), and set **Coloring Mode** to **By Slice**. Click the **Set** button.

In the COLOR PEAKS BY PLANE control panel, leave the default values and click **OK**. Click the **Plot** icon from the tool bar to redraw the spectrum.

Click the **Real-Time Plane Selection** icon. Move the slider in the control panel to step through the planes.

The peaks are displayed in different colors, depending on whether they are centered on the current plane or not.

9. Plotting 2D slices of the 3D spectrum

Select the **View/Limits/Select Plane** menu item. In the control panel, set the **Unit** to **points** and set **D1_H1-D3_N15 at D2_C13** to **32**.

Click **OK**.

*Now you have D1 as the horizontal axis and D3 as the vertical axis. The number of the D2 slice, 32 (or the chemical shift value if you have selected **PPM** as the axis unit) is shown in the lower-right corner.*

*You can use the **Preference/Plot Parameters** menu item to adjust the attributes of the plot, if necessary, and to select the appropriate display parameters, such as **Contour Threshold**.*

Next you learn to page through the 2D slices interactively.

If the **Real-time Plane** control is not displayed yet, click the **Real-time Plane Selection** icon to display it. Click the slider in the **Real-time Plane** control and move it, then release it. You can also step through the planes by pressing the left or right arrow key when the slider is highlighted.

This updates the contour plot. You can also type in a new plane via ***plane number*** *or by using the* ***ppm*** *box*

10. Exiting FELIX

At this point you may exit FELIX by selecting the **File/Exit** menu item.

4 Using Assignment

Two crucial steps in structure determination using NMR spectra are to assign each nucleus to a specific chemical shift (so-called sequence-specific assignment) and to assign each peak in relevant spectra to these assigned resonances. These two steps together constitute the assignment procedure. Three example lessons are presented in the following section, which highlight the basic steps.

- *Lesson 1: Homonuclear 2D assignment strategy*—illustrates the basic steps involved in assigning a medium-sized protein using homonuclear 2D spectra.
- *Lesson 2: Heteronuclear double-resonance 3D assignment strategy*—teaches you to make sequence-specific assignments using the ^{15}N double resonance spectrum of a protein.
- *Lesson 3: Heteronuclear triple resonance 3D assignment strategy*—focuses on conducting a spin-system detection and assignment using the heteronuclear triple resonance spectrum of a protein.

Lesson 1: Homonuclear 2D assignment strategy

This lesson presents the basic steps of an NMR spectrum assignment using homonuclear spectra, including TOCSY, DQF-COSY, and NOESY 2D NMR spectra of Zn–rubredoxin (Blake et al. 1991).

The topics covered in this lesson are:

- Database setup.
- Spin-system detection.
- Spin-system connection and identification.
- Sequence-specific assignment of spin systems.
- Automated peak assignment.
- Restraint generation.

- Export of databases.
- Restraint redefinition.
- Chemical shift index calculation.

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*. The files for this lesson are located in the **Assignment\Lesson1** folder.

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**. If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, navigate to your working directory. If you used the default suggestion, this will be: **C:\Felix_Practice\Assignment\Lesson1**.

Enter **zn.dba** and click **OK** to build a new database file.

3. Going to the Assign module

Select the **Assign/Project** item from the menubar.

4. Setting up the database

When FELIX informs you that no project was found, click **OK**. FELIX will prompt you for a new project.

In the control panel, the default name of the project appears as **asg:project**. You can enter another name if you want (e.g., **zn:project**).

In the next control panel, select the linear chain of the molecule by setting **File name** to **znrdlec.car**.

Click **OK** to build the entities and read in the molecule.

This procedure typically takes several seconds. Then the program asks for the library. The library is an ASCII file, as described in "Assign/Define Library" in Chapter 5, Assign User Interface, in the FELIX User Guide. FELIX contains a standard library for proteins and DNA (pd.rdb) which you should read in.

In the next control panel, select the **Define Library from File** option and click **OK**.

In the following control panel, select **pd.rdb**.

This is the protein/DNA library. A few seconds later the project setup procedure finishes.

5. Viewing the project entity through a spreadsheet

Select the **Assign/Project** menu item again.

The project entity is presented in the spreadsheet, and you can browse through its fields.

Many fields contain zeros or nulls, since the full definition is not finished yet. There are nine experiment columns, therefore you can define nine experiments in one project.

Select **File/Close** in the table to exit the spreadsheet.

6. Adding an experiment to the projects

Select the **Assign/Experiment** menu item to define new experiments in the assignment database. Select **zc.mat** (the **DQF-COSY** spectrum).

Click **OK**.

Set the following parameter values for the plot using the 2D Display Parameters control panel:

Contour Threshold	**0.005**
Color Scheme	**Blue/Green**
Axis type D1	**ppm**
Axis type D2	**ppm**

Leave the other parameters at their default values and select **Apply**.

Click **OK** when the message box appears.

*If you want, you can change the display parameters using the **Experiment/Change Attribute** menu item in the **Experiments** table.*

The program plots a density or contour plot of the DQF-COSY using the parameters you defined. The coloring scheme is a predefined blue and green colormap with 16 blue colors for positive peaks and 16 green colors for negative peaks.

What you enter for **Experiment Title** should be descriptive, but not too long (for example, **COSY** or **DQF** is appropriate for this spectrum). Leave **Use Default Names** toggled **on** (which automatically fills in the peak table, volume table, and J table names). Set the remaining parameters to these values:

Type	**2D DQF**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Proton**
D2 Nucleus	**Proton**
D1 Tol	**0.008**
D2 Tol	**0.008**
W1	**D2**
W2	**D1**
W1-W2 Transfer	**J-coupled**
# of J Steps	**3**

Click **OK**.

It is important to define the spectrum-specific tolerances, which are used in many automated and semi-automated procedures.

7. Repeating Step 6 for the TOCSY and NOESY spectra

Select the **Project/Experiment** menu item again.

This brings up a spreadsheet with the currently-defined experiments. You can use this spreadsheet to add, delete, or edit experiments.

Now go to the spreadsheet menubar and select the **Experiment/Add** item.

When the control panel appears, select **zh.mat** for **TOCSY** and **zn.mat** for **NOESY**. The required values for each run are different:

Parameter Name	TOCSY Value	NOESY Value
Contour Threshold	**0.015**	**0.01**
Color Scheme	**Magenta**	**Cyan**
Number Of Levels	**10**	**10**
Negative Levels	**Off**	**Off**
Axis Type D1	**ppm**	**ppm**
Axis Type D2	**ppm**	**ppm**

Leave the remaining parameters at their default values and click **OK**.

In the next control panel, set these parameter values for TOCSY:

Parameter Name	TOCSY Value	NOESY Value
Experiment Title	**tocsy**	**noe**
Use Default Name	**on**	**on**

Type	2D TOCSY	2D NOESY
D1 Nucleus	Proton	Proton
D2 Nucleus	Proton	Proton
W1	D2	D2
W2	D1	D1
W1-W2 Transfer	J-coupled	NOE

Number of J Steps	7	0
Mixing Time	0	0.05
D1 Tolerance	0.008	0.01
D2 Tolerance	0.008	0.01

Leave the remaining parameters set to their default values.

8. Checking the project entity

You may need to highlight the spectral window if the main menu is not displayed. Next select **Assign/Project** to display the Project table. You can click the maximize button in the upper right corner of the table to expand it for a better view. After reviewing the items in the table, select **File/ Close** to close it.

Note that previously zero or null fields now have values.

9. Drawing the full DQF-COSY spectrum

If necessary, select **Window/Auto Arrange** to re-arrange the Experiment table and the spectral window.

Now go to the **Experiments** table and select the **cosy** spectrum by clicking the first row and then clicking the **Draw** icon.

The next step in the assignment procedure is to do a peak picking. This procedure is very important, since all other steps rely on proper peak picking.

Usually peak picking involves several steps. First the automatic peak picker should be run. You can run the regular peak picker or the Stella peak picker. The results are then filtered automatically (symmetrizing, deleting the diagonals, deleting artifacts (solvent ridges), and deleting peaks with invalid widths). You should also thoroughly inspect the results visually, to ensure there is enough confidence in the data. FELIX also provides a tool to fit the 2D peaks via the ***Peaks/Optimize*** *menu item (see Peaks/Optimize on page 146 in Chapter 4, Processing, Visualization, and Analysis Interface (1D/2D/ND), in the FELIX User Guide), which also increases the accuracy of peak picking.*

Note: The importance of peak picking cannot be overemphasized, since the automated assignment tools work only as well as the starting conditions permit ("garbage in garbage out"). Bearing this in mind, Accelrys has tried to give you a clean peak set. Therefore you need to read this peak set from the text directory provided (dqf.xpk, tocsy.xpk and noe.xpk).

10. Reading in the peaks

From the main menu, select the **File/Import/Peaks** menu item. Set the parameter **Peak File Type** to **FELIX Peak File(*.*)**. Select **dqf.xpk** as File name. Leave **FELIX Peak Table Name** at its current setting (**xpk:cosy**), since your current experiment is a DQF-COSY.

Click **OK**.

When the query box appears, asking about overwriting the entity, select **Overwrite**.

The footprints of the imported peaks are displayed on the DQF-COSY spectrum. You may need to click the Plot icon from the main tool bar to

redraw the spectrum. A Peaks-xpk:dqf table is also displayed, showing the imported peaks.

Go to the Experiment table and select the TOCSY spectrum. Click the Draw icon to display it.

From the main menu select the **File/Import/Peaks** menu item. Set the **Peak File Type** to **FELIX Peak Type(*.*)**. Select **tocsy.xpk** as **File name** and click **OK**.

When the query box appears, asking about overwriting the entity, select **Overwrite**.

Select **File/Close** from the Peaks-xpk:tocsy table menu to close the table.

Next you repeat the procedure for the NOE spectrum.

Select the NOE spectrum in the **Experiments** table and click the **Draw** icon to display it.

From the main menu, select the **File/Import/Peaks** menu item. Set the **Peak File Type** to **FELIX Peak Type(*.*)**. Select **noe.xpk** as **File name**. Leave **FELIX Peak Table Name** as **xpk:noe** and click **OK**.

When the query box appears, asking about overwriting the entity, enter **Overwrite**.

Select **File/Close** from the **Peaks-xpk:noe** table menu to close the table.

Now you have a full peak set defined for all three experiments.

11. Selecting the DQF-COSY spectrum

Select the **DQF-COSY** spectrum in the **Experiments** table and click the **Draw** icon. Highlight the spectral window and if necessary, press <Ctrl>-f on your keyboard to obtain the full plot.

*The displayed footprints belong to this spectrum, not to the **NOESY**, which was read in last. The database took care of reloading the spectrum-specific information.*

*The next step is the collection of prototype patterns, i.e., sets of frequencies, which later are promoted to patterns and assigned to specific amino acid residues. The menu items relating to prototype patterns are in the third subsection of the **Assign** pulldown. First we demonstrate a method which uses all three available (COSY, TOCSY, and NOESY) spectra to generate prototype patterns.*

12. Performing a prototype pattern detection

Select the **Assign/Collect Prototype Patterns** menu item. From the control panel select the **2D Homonuclear** option and click **OK**.

In this tutorial the homonuclear 2D spectra are used for assignment.

In the subsequent control panel, set **Spin System Type** to **Proteins** and **Systematic Search** to **Method**. Click **OK**.

A control panel with several options appears. The program tries to fill in reasonable values.

Set these parameter values in the control panel:

Method:	**COSY+TOCSY+NOE**
COSY experiment:	**cosy**
TOCSY experiment:	**tocsy**
NOE experiment:	**noe**
Seed/Expansion:	**Use Defaults**
Frequency Collapse Tolerance:	**0.015**
Output level:	**Low**

*The **Frequency Collapse Tolerance** is the tolerance for aligning and finding connected expansion peaks with seed peaks.*

At this point you could just start the collection and use the defaults for the seed peak and expansion peak area. You can also look at them by clicking the **More...** button instead of the **OK** button. In the next control panel leave all parameters at the default values:

Seed area D1	
Low	**6**
High	**12**
Seed area D2	
Low	**3**
High	**5.5**
Expansion area D1	
Low	**-2**
High	**12**
Expansion area D2	
Low	**-2**
High	**12**
Remove Intraproto Frequencies on Number of Frequencies in Proto	
Min	**3**
Max	**8**
Number of Iterations	**6**
Frequencies Per Iteration	**1**

*The **Seed Area D2 (High)** is the amide proton region above the diagonal. **Remove Intraproto Frequency on Number of Frequencies in Proto** is the minimum and maximum number of frequencies in a prototype pattern. **Number of Iterations** is the maximum number of expansion loops, and **Frequencies Per Iteration** is the number of frequencies in each loop to keep.*

In the remaining part of the control panel, set these parameter values:

# ppm filters active	**2**
Filter #	
1	
Low	**6**
High	**12**
Min	**1**
Max	**1**
2	
Low	**3**
High	**5.5**
Min	**1**
Max	**3**

Click **OK**.

Only those prototype patterns are kept which have at least (and at most) one frequency in the 6–12 ppm region (amide proton) and at least one (and at most three) frequencies in the 3–5.5 ppm region.

*Be sure to leave the **Min # cont** (the minimum number of contacts) values at their defaults (**1 1 2 2**, **1 2 2 3**, and **2 2 3 4**).*

In the output window, information is displayed about the current stage of prototype pattern collection. After one minute, the prototype pattern collection is finished for 106 seed peaks and 3240 expansion peaks, and the following information appears in the output window:

```
   Nr of prototype patterns generated:(57)
The 2D protopattern detection took 7 seconds
```

*Also, a spreadsheet containing the prototype patterns is displayed (**Protopatterns**).*

13. Saving the results of prototype pattern detection as a file

Highlight the Protopatterns table and select the **File/Save As** menu item. Set the **File name** as **zn_protos.txt** and click **OK**.

In the output window you are informed about the success of the command:

```
Saved table:table 'Protopatterns' (entity zn:proto)as File
'C:\tutorial\Assignment\Lesson1\zn_protos.txt'.
```

*The next step is to visually inspect the prototype patterns. The **Protopatterns** spreadsheet provides several ways for you to see prototype patterns: you can draw frequencies of prototype patterns as lines on top of a contour plot, spawn tiles, or draw a strip plot.*

Go to the **Protopatterns** table and select the **Preferences/ Draw** menu item.

When the control panel appears, set **Vertical Color** to **Blue** and **Horizontal Color** to **Green**. Click **OK**.

Now click the first row of the table (select the first prototype pattern) and click the **Draw** icon.

You see four lines at 9.7, 5.37, 1.78, and 0.89 ppm, which are frequencies in this prototype pattern.

Right-click in the spectral window and select the **Clear Frequencies** command in the context menu to clean the frequency lines.

The second way to visualize prototype patterns is to spawn tile plots from them. This allows you to concentrate only on frequencies and peaks belonging to them, which are present in this prototype pattern.

14. Making a tile plot of prototype pattern 1

Reselect the first prototype pattern from the table and click the **Tile Plot** icon. If you want to change the tile plot attributes, go to the table and select the **Preferences/Tile Plot** menu item.

The tile plot is displayed.

Highlight the spectral window if necessary, and press <Ctrl>-c (if you were in intensity-plot mode) to see the contour plot of the **COSY** spectrum tiled by the first prototype pattern.

Select the tocsy spectrum from the Experiments table and click **Draw**.

Using the tile plot functionality, you can concentrate on peaks and their immediate surroundings which belong to a prototype pattern. Also, you can use strip plots to see strips surrounding the frequencies in vertical or in horizontal position.

15. Returning from tile mode

While the spectral window is highlighted, press <5> in the keypad and use the large cross-hair cursor to pick one of the boxes. This command (**Jump**) places only that small region on the screen and exits tile-plot mode.

Go to the Protopatterns table and select the **Preferences/ Strip Plot** menu item. Set these parameters:

Shift Type	**Generic**
Dimension	**W2**
Width	**64**
Scale	**4.**

Click **OK**.

Click the **Strip Plot** icon in the table.

You see four vertical strips with the frequencies of the first prototype pattern in the middle of each.

From the strip plot you can see that there are no outstanding peaks that have common chemical shifts with the frequencies in this prototype pattern. Therefore you can continue to promote this prototype pattern to pattern. The first step in this procedure is to copy these frequencies to the clipboard.

16. Copying a prototype pattern to the frequency clipboard

While the spectral window is highlighted, select the **Assign/ Frequency Clipboard/Copy Proto to Clipboard** menu item. In the control panel, select **1** as the **Proto Pattern** and click **OK**.

The first prototype pattern is now copied to the clipboard list. This list can be manipulated (you may add or delete frequencies to or from the list, swap the order of two frequencies, delete duplicate frequencies, sort the list, or zero the list). You can also display the list as lines on top of the matrix plot or spawn a tile and strip plot from it.

Select the **Assign/Frequency Clipboard/Sort Clipboard** menu item. Now you can sort the frequencies in the clipboard in descending ppm order by toggling **Descending order** to **on**.

Next select the **Assign/Frequency Clipboard/View Clipboard** menu item to view the content in the clipboard.

The results should look like this:

```
The Frequency Clipboard List contains the following frequencies:

    # Freq(ppm) Atom

--- --------- ----

 1   9.703     X
 2   5.369     X
 3   1.786     X
 4   0.895     X
```

If there is no appropriate frequency to add or delete, the clipboard list can be promoted to a pattern, and the pattern can be then subjected to database searches and naming.

17. Copying the frequency clipboard to the pattern

Select the **Assign/Frequency Clipboard /Copy Clipboard to Pattern** menu item. In the control panel, keep all the default settings and click **OK**.

*Now the four frequencies in the clipboard are promoted to a spin system. Also, a new spreadsheet is displayed—**Spinsystems**. You can list this pattern to a file or to the output window or you can examine it in the spreadsheet. Also, you can close this spreadsheet and reopen it using the **Edit/Spin Systems** menu item.*

18. Viewing the pattern

While the spectral window is activated, select the **Assign/ Report Spin System** menu item. Make sure that **Action** is set to **To Textport** and click **OK**.

This prints the following information to the output window:

```
Listing for pattern: pa1
 comment : null
 color   : Red
 root frequency: 9.703

                        Frequencies
                    -----------
 generic              specific                    assignment
 shift                shifts
1     2     3     4     5     6     7     8     9
           cosy tocsy noe null null null null null null
 9.703     9.703 9.703 9.703 9.703 9.703 9.703 9.703 9.703 9.703     1:*_*:HX
 5.369     5.369 5.369 5.369 5.369 5.369 5.369 5.369 5.369 5.369     1:*_*:HX
 1.786     1.786 1.786 1.786 1.786 1.786 1.786 1.786 1.786 1.786     1:*_*:HX
 0.895     0.895 0.895 0.895 0.895 0.895 0.895 0.895 0.895 0.895     1:*_*:HX

The neighbor probabilities
--------------------------
 Pattern                  Probability

The residue type probabilities
 ------------------------------
Residue                   Probability
```

Next you copy the generic shifts for the frequencies to the spectrum-specific category. Since these chemical shifts were detected in the TOCSY spectrum, you can copy this to the experiment without any change.

19. Finding the residues that the pattern belongs to

Select the **Assign/Residue Type/Score Residue Type** menu item. Toggle off **Use All Patterns**. Select the **pa1** pattern, set the **Max Std Dev** to **3**, and set the **Min Atoms** to **Specify** and **4**. Set the **Scoring method** to **All Atoms**, and **Database** to **Store**. Click **OK**.

In the output window, a report is generated of the probabilities that this pattern belongs to a certain type of amino acid residue:

```
Scoring pattern pa1
 9.703 5.369 1.786 0.895
 'H' 'H' 'H' 'H'
 ... vs ile | score=1 | aver.=1.249352 | matched atoms=4 / 4
 ... vs leu | score=1 | aver.=2.018772 | matched atoms=4 / 4
 ... vs lys | score=1 | aver.=1.940873 | matched atoms=4 / 4
 ... vs thr | score=0.8 | aver.=1.6740694 | matched atoms=4 / 5
 ... vs val | score=1 | aver.=1.569354 | matched atoms=4 / 4
Scoring is done.
```

Since the highest score and lowest average is for the Ile and Val residues, since you have seen from strip plots that there are no extra resonances, and since Ile theoretically has seven resonances, while Val has only five (with methyl degeneracy likely four), you can now assume that pattern pa1 is a valine type.

Generally, the probability is higher if the score higher and the average is lower. The best-matched atoms give a higher confidence in the probability. You can store the result with the same control panel, by selecting the ***Store Result*** *option.*

You can try to perform this action again, using the DQF-COSY peaks to help distinguish between equally likely residue types. If you do, the printout will be:

```
Scoring pattern pa1
 9.703 5.369 1.786 0.895
 'H' 'H' 'H' 'H'
 ... vs ile | score=0.375 | aver.=1.249352 | matched atoms=4 / 4 | cosy=3 / 8
 ... vs leu | score=0.25 | aver.=2.018772 | matched atoms=4 / 4 | cosy=2 / 8
 ... vs lys | score=0.0526316 | aver.=1.940873 | matched atoms=4 / 4 | cosy=1 / 19
 ... vs thr | score=0 | aver.=1.670694 | matched atoms=4 / 5 | cosy=0 / 3
 ... vs val | score=0.75 | aver.=1.569354 | matched atoms=4 / 4 | cosy=3 / 4
 Scoring is done.
```

This can help in further distinguishing residue types. After you decide that this pattern is a valine type, you need to see which frequency belongs to which atom. For this you must query the database.

20. Querying the database

Select the **Assign/Residue Type/Match Residue Type** menu item. Select **pa1** as the **pattern** and select the **Val** residue to match against it. Click **OK**.

The result is a table in the output window showing the relative differences of each frequency from its expectation value. The smallest absolute value shows the highest matching:

```
Matching pattern pa1 versus val
          9.703   5.369   1.786   0.895
        H H H H
HN        2.579  -4.526 -10.400 -11.861
HA       10.187   2.307  -4.207  -5.827
HB       30.572  13.236  -1.096  -4.660
HG1*     40.332  20.632   4.345   0.295
HG2*     48.906  24.828   4.922  -0.028
```

You can see that the frequency with 9.703 ppm probably belongs to the H_N resonance and that the H_α is the frequency with 5.369 ppm. H_β is the frequency with 1.786 ppm, The two gamma methyls are not resolved, but the 0.895 ppm frequency belongs to them. You need to set these findings in your database. To do this, you must assign these frequencies.

21. Assigning the frequencies

Select the **Assign/Assign Spin System/Frequency** menu item. Select **pa1**, and click **Next**. This brings up a control panel in which you can make the assignment. Choose the first frequency **9.703** in the **Frequency** list and click the **Select** button. Select the **VAL** item from the **Residues** list and click the **Filter** button. This fills in the **#S** list with **4**, **37**, and *****.

Since you do not know which valine this pattern belongs to, choose the wild card (*****), then select the **HN** item from the **Nuclei** list. Click the **Build** button, which fills in the **Atom Spec** with **1:VAL_*:HN**. Now click **Add**. Since the database contains only atoms that belong to certain residues and the selected residue is not a real one, a dialog box appears asking you for confirmation to include this “new atom” in the database. Click **Yes**.

Now repeat the procedure for the other frequencies in this pattern. When you are finished, select **Quit**.

You can see the results of this assignment by using the **Assign/Report Spin System** menu item.

```
LISTING FOR PATTERN pa1

 comment            :  null
 color              :  Red
 root frequency     :  9.703

                      Frequencies
                      -----------
 generic               specific                    assignment
  shift                 shifts
             1     2     3     4     5     6     7     8     9
           cosy tocsy noe null null null null null null
 9.703     9.703 9.703 9.703 9.703 9.703 9.703 9.703 9.703 9.703     1:VAL_*:HN
 5.369     5.369 5.369 5.369 5.369 5.369 5.369 5.369 5.369 5.369     1:VAL_*:HA
 1.786     1.786 1.786 1.786 1.786 1.786 1.786 1.786 1.786 1.786     1:VAL_*:HB
 0.895     0.895 0.895 0.895 0.895 0.895 0.895 0.895 0.895 0.895     1:VAL_*:HG1*

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      LYS                 1.000
      VAL                 1.000
      ILE                 1.000
      THR                 0.800
      LEU                 1.000
```

*Since the frequencies were defined from the **TOCSY** experiment in the pattern, you need to edit the NOE and DQF frequencies. For this, use the **Assign/Spin System/Tile+Show+Edit Frequencies** menu item.*

22. Adjusting the spectrum-specific shifts for NOE

First you need to define the NOE spectrum-specific shifts for frequencies.

Select from the **Experiments** table the NOE spectrum and click the **Draw** icon.

Now select the **Assign/Spin System/Tile+Show+Edit Frequencies** menu item. Choose **pa1** from the **Pattern List** and select **Specific Shift** and **noe**. Click **OK**.

*You see the spectrum-specific shifts drawn on top of the tiled plot of the **NOE** spectrum, and an instruction message in the status bar (as well as in the title of the main window):*

```
Click-drag-release to include the frequency you want to
edit, or click <ESC> to quit.
```

Suppose the frequency 0.895 does not coincide with the NOE peaks well and you want to edit this frequency for the NOE spectrum. You may edit this frequency:

In any of the tiles that contain the 0.895 ppm frequency (e.g., the upper left-most tile), click-drag to draw a small rubber box that overlaps with the frequency 0.895 ppm. Release the mouse button. This selects the 0.895 ppm frequency for editing, and the cursor becomes a horizontal hair.

A new instruction message appears in the status bar (as well as in the title of the main window):

```
Pick on the new position or hit <ESC> to quit.
```

Move the horizontal hair cursor to a location that aligns best with the NOE peaks. Click the left mouse button to select it.

The new shift is displayed, and a message appears with the new chemical shifts. You may have something similar to:

```
Selected frequency(s) to change:

D2:  0.892

Changed to new frequency(s):

D2:  0.9150143

Applied the changes to frequencies of experiment 'noe'.
```

You may repeat these steps for each frequency to obtain the best alignment for each frequency.

After you finish with the NOESY spectrum, you can repeat this for the DQF-COSY spectrum and check the results with the **Assign/Report Spin System** menu item.

You should notice that, under Frequencies, the specific shifts for NOE have been changed.

You now need to inspect the other prototype patterns and promote them to patterns, as was done in Steps 16, 17, and 18.

23. Copying the 55th prototype pattern to the clipboard list

Select **Assign/Frequency Clipboard/Zero Clipboard** to clear the frequency clipboard. Next select **Assign/ Frequency Clipboard/Copy Proto to Clipboard** and copy prototype pattern **55**.

If TOCSY is not currently displayed, select it in the Experiment table and click the **Draw** icon. Next activate the spectral window and select the **Assign/Frequency Clipboard/ Strip Plot Clipboard** menu item to display the strip plot.

You can see that, in the column of 9.194 ppm, there is an extra frequency around 2.5 ppm.

Use the **Zoom** icon in the main tool bar to zoom to the (9.19, 2.5 ppm) peak region.

Add this 2.5 ppm frequency to the frequency clipboard by selecting the **Assign/Frequency Clipboard/Add One** menu item, clicking the cursor on this peak, and then setting the **Frequency** parameter to **W1 2.664909** and the **Nucleus 1** to **HX**. Click **OK**.

Click <ESC> to terminate the frequency adding mode. Select **Assign/Frequency Clipboard/Copy Clipboard** to Pattern. Leave all the default settings in the dialog box and click **OK**. This promotes the frequencies in the clipboard to a new pattern, **pa2**.

Score the pattern as in Step 19. The result is:

```
Scoring pattern pa2
 9.194 3.060 2.845 2.665
 'H' 'H' 'H' 'H'
 ... vs cys | score=1 | aver.=1.168145 | matched atoms=4 / 4
 ... vs glu | score=0.6666667 | aver.=2.168036 | matched atoms=4 / 6
 ... vs lys | score=0.4444444 | aver.=1.916762 | matched atoms=4 / 9
 ... vs ser | score=1 | aver.=2.377159 | matched atoms=4 / 4
 ... vs tyr | score=0.3636364 | aver.=0.464548 | matched atoms=4 / 11
 Scoring is done.
The most probable amino acid residue type is cysteine. Match this pattern against
CYS:
Matching pattern pa2 versus cys
            9.194    3.060    2.845    2.665
          H H H H
 HN         1.277   -7.486   -7.793   -8.050
 HA         6.125   -2.053   -2.340   -2.580
 HB1       16.668    0.526   -0.039   -0.513
 HB2       15.879   -0.263   -0.829   -1.303
```

If you check the DQF-COSY spectrum (by selecting to display the COSY from the Experiment table, and then selecting pa2 in the Spin-systems table and clicking the Tile Plot icon), you can see that the frequency with the chemical shift of 2.845 has a cross peak with an amide proton (9.194 ppm), therefore this must be the alpha proton. HB2 is probably the frequency with 3.060 ppm, and HB1 the frequency with 2.665 ppm. Assign this pattern as described in Step 21. The result should be similar to:

```
LISTING FOR PATTERN pa2

 comment            :  null
 color              :  Red
 root frequency     :  9.194

                      Frequencies
                      -----------
 generic               specific                    assignment
  shift                 shifts
             1     2     3     4     5     6     7     8     9
           cosy tocsy noe null null null null null null
 9.194      9.194 9.194 9.194 9.194 9.194 9.194 9.194 9.194 9.194     1:CYSH_*:HN
 3.060      3.060 3.060 3.060 3.060 3.060 3.060 3.060 3.060 3.060     1:CYSH_*:HB2
 2.845      2.845 2.845 2.845 2.845 2.845 2.845 2.845 2.845 2.845     1:CYSH_*:HA
 2.665      2.665 2.665 2.665 2.665 2.665 2.665 2.665 2.665 2.665     1:CYSH_*:HB1

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      LYS                 0.444
      CYS                 1.000
      TYR                 0.364
      GLU                 0.667
      SER                 1.000
```

24. Copying the 52nd prototype pattern to the clipboard list

Clear the clipboard using the **Assign/Frequency Clipboard/Zero Clipboard** menu item, then copy the 52nd prototype pattern to the clipboard with the **Assign/Frequency Clipboard/Copy Proto to Clipboard** menu item. Spawn a tile for the TOCSY spectrum as in Step 14, but use the clipboard as the source (**Assign/Frequency Clipboard/Tile Clipboard**).

You can see that, in the left-most tile in the row with **1.983 ppm**, there is an extra frequency around **1.89 ppm**.

Add this frequency to the clipboard list with the **Assign/Frequency Clipboard/Add One** menu item. Copy this clipboard list to the pattern **pa3** and score it with **Min atoms** set to **6**.

You should get the following output:

```
Scoring pattern pa3
 8.928 3.972 1.983 1.498 1.674 1.893
 'H' 'H' 'H' 'H' 'H' 'H'
... vs leu | score=1 | aver.=1.084693 | matched atoms=6 / 6
... vs lys | score=1 | aver.=0.6165203 | matched atoms=6 / 6
 Scoring is done.
```

The most probable amino acid residue type is lysine. Select **Assign/Residue Type/Match Residue Type** to match against it, and then assign it.

```
Matching pattern pa3 versus lys
            8.928    3.972    1.983    1.498    1.674    1.893
          H H H H H H
 HN         0.997   -6.628   -9.688  -10.434  -10.163   -9.826
 HA        11.210   -0.590   -5.326   -6.481   -6.062   -5.540
 HB1       18.942    5.900    0.666   -0.611   -0.147    0.429
 HB2       20.935    6.359    0.509   -0.918   -0.400    0.244
 HG1       19.277    6.569    1.469    0.226    0.677    1.238
 HG2       20.265    6.870    1.495    0.184    0.659    1.251
 HD1       30.242    9.592    1.304   -0.717    0.017    0.929
 HD2       31.513    9.965    1.317   -0.791   -0.026    0.926
 HE1       45.754    7.631   -7.669  -11.400  -10.046   -8.362
 HE2       59.380    9.820  -10.070  -14.920  -13.160  -10.970
 HZ*        1.398   -3.558   -5.547   -6.032   -5.856   -5.637
```

*Further inspecting the TOCSY spectrum (strip plots), you can see two extra frequencies, at around 2.99 and 2.88 ppm, which you can add to the pattern with the **Assign/Spin System/Add Frequency via Cursor** menu item.*

Now score this pattern again and store the result. Use **8** as the **Min Atoms**.

The result should be similar to:

```
Scoring pattern pa3
 8.928 3.972 1.983 1.498 1.674 1.893 2.885 2.995
 'H' 'H' 'H' 'H' 'H' 'H' 'H' 'H'
... vs lys | score=0.8 | aver.=0.6080632 | matched atoms=8 / 10
Scoring is done.
```

which clearly shows that the original assumption—that the pattern is a listen type—was a valid one (leucine is now ruled out, although it was possible from the score itself).

Unambiguous assignment is possible only for the amide and alpha proton, therefore the pattern listing will show the following results:

```
LISTING FOR PATTERN pa3

 comment             :  null
 color               :  Red
 root frequency      :  8.928

                       Frequencies
                       -----------
 generic                specific                      assignment
  shift                  shifts
              1      2      3      4      5      6      7      8      9
            cosy tocsy noe null null null null null null
 8.928      8.928 8.928 8.928 8.928 8.928 8.928 8.928 8.928 8.928      1:LYS+_*:HN
 3.972      3.972 3.972 3.972 3.972 3.972 3.972 3.972 3.972 3.972      1:LYS+_*:HA
 1.983      1.983 1.983 1.983 1.983 1.983 1.983 1.983 1.983 1.983      1:LYS+_*:HX
 1.498      1.498 1.498 1.498 1.498 1.498 1.498 1.498 1.498 1.498      1:LYS+_*:HX
 1.674      1.674 1.674 1.674 1.674 1.674 1.674 1.674 1.674 1.674      1:LYS+_*:HX
 1.893      1.893 1.893 1.893 1.893 1.893 1.893 1.893 1.893 1.893      1:LYS+_*:HX
 2.995      2.995 2.995 2.995 2.995 2.995 2.995 2.995 2.995 2.995      1:LYS+_*:HX
 2.885      2.885 2.885 2.885 2.885 2.885 2.885 2.885 2.885 2.885      1:LYS+_*:HX

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      LYS                 0.800
```

25. Copying the 49th prototype pattern to the frequency clipboard

Follow the procedure described in Step 25 to copy prototype pattern 49 to the clipboard. Then spawn a tile plot from the clipboard for the TOCSY spectrum using the **Assign/ Frequency Clipboard/Tile Clipboard** menu item. You can see that a frequency at 0.853 ppm was missed during the automated routine. Add it to the clipboard and then copy the list to the **pa4** pattern. Set the spectrum-specific shifts. Now score and store the result of the pattern using **5** as the **Min Atoms**:

```
Scoring pattern pa4
 9.094 4.049 2.545 1.366 0.846 0.749
 'H' 'H' 'H' 'H' 'H' 'H'
... vs ile | score=0.7142857 | aver.=1.097882 | matched atoms=5 / 7
 ... vs leu | score=1 | aver.=1.303445 | matched atoms=6 / 6
 ... vs lys | score=1 | aver.=1.344301 | matched atoms=6 / 6
 ... vs pro | score=0.7142857 | aver.=1.726693 | matched atoms=5 / 7
 ... vs val | score=0.8333333 | aver.=0.9049344 | matched atoms=5 / 5
 Scoring is done.
```

Since there are clearly at least six frequencies in the pattern, the valine possibility can be dropped. Also, since there is an H_N frequency, the proline can be excluded. The remaining possibilities are leucine, lysine, and isoleucine. Since the frequencies with 0.836 and 0.735 ppm are methyl groups, the lysine can also be excluded.

From the Experiments table, select the **DQF** spectrum. Click the **Draw** icon to display the COSY spectrum.

From this you can see that the frequency with 2.545 ppm belongs to a possible beta methine or methylene proton. This is connected with a strong COSY interaction with the methyl frequency at 0.846 ppm, which is only possible in an isoleucine spin system. Therefore, this spin system is an isoleucine type.

In the **Spinsystems** table, select the **Spinsystem/List Residue Type** menu item.

Select the **Assign/Residue Type/Set Residue Type** menu item for pa4 and choose **Assign One** as the **Action**. Choose **ILE** from the list and click **OK**.

A message appears:

```
Residue type of pattern pa4 is set to ile
```

You can verify the results with the **Assign/Residue Type/ Show Residue Type** menu item.

The output is:

```
The probability for pa4 to be ILE is : 1.000
```

*After matching the pattern against the **ILE** residue, you can assign the frequencies.*

```
Matching pattern pa4 versus ile
            9.094   4.049   2.545   1.366   0.846 0.749
          H H H H H H
 HN         1.283  -5.724  -7.812  -9.450 -10.163 -10.307
 HA         9.431  -0.271  -3.163  -5.431  -6.417  -6.617
 HB        19.795   6.159   2.095  -1.092  -2.478  -2.759
 HG11      30.977  11.573   5.788   1.254  -0.719  -1.119
 HG12      23.981   8.216   3.516  -0.169  -1.772  -2.097
 HG2*      34.600  13.579   7.312   2.400   0.262  -0.171
 HD1*      33.416  13.236   7.220   2.504   0.452   0.036
```

Since you saw from the DQF spectrum that the frequency with 2.545 ppm is the beta methine proton and that it has a cross peak with the methyl at 0.846 ppm, the assignments are as follows:

```
LISTING FOR PATTERN pa4

 comment            :  null
 color              :  Red
 root frequency     :  9.094

                      Frequencies
                      -----------
 generic               specific                      assignment
  shift                 shifts
              1     2     3     4     5     6     7     8     9
            cosy tocsy noe null null null null null null
 9.094      9.094 9.094 9.094 9.094 9.094 9.094 9.094 9.094 9.094     1:ILE_*:HN
 4.049      4.049 4.049 4.049 4.049 4.049 4.049 4.049 4.049 4.049     1:ILE_*:HA
 2.545      2.545 2.545 2.545 2.545 2.545 2.545 2.545 2.545 2.545     1:ILE_*:HB
 1.366      1.366 1.366 1.366 1.366 1.366 1.366 1.366 1.366 1.366     1:ILE_*:HG12
0.84653      0.853 0.853 0.853 0.853 0.853 0.853 0.853 0.853 0.853     1:ILE_*:HG2*
0.749      0.749 0.749 0.749 0.749 0.749 0.749 0.749 0.749 0.749     1:ILE_*:HD1*

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      ILE                 1.000
```

26. Copying the 4th prototype pattern to clipboard list

This time you will promote (copy) a prototype pattern directly to a pattern (spin system).

Select the 4th prototype pattern in the **Protopatterns** table by clicking the fourth row.

Go to the table menubar and select the **ProtoPattern** / **Promote One Proto To Spin System** item.

In the control panel,, leave all the default settings unchanged. Click **OK**.

This adds a new spin system, pa5, in the Spinsystems table.

Select **Assign/Residue Type/Score Residue Type** to score and store the result of pattern **pa5**. Use **5** as the **Min Atoms:**.

The residue type scoring appears in the output window as:

```
Scoring pattern pa5
 9.274 5.022 3.288 2.526
 'H' 'H' 'H' 'H'
 ... vs asn | score=1 | aver.=1.229159 | matched atoms=4 / 4
 ... vs asp | score=1 | aver.=1.165284 | matched atoms=4 / 4
 ... vs cys | score=1 | aver.=0.7924712 | matched atoms=4 / 4
 ... vs lys | score=0.4444444 | aver.=1.975681 | matched atoms=4 / 9
 ... vs phe | score=1 | aver.=0.78622 | matched atoms=4 / 4
 ... vs ser | score=0.8 | aver.=1.212075 | matched atoms=4 / 5
 ... vs thr | score=0.8 | aver.=1.514605 | matched atoms=4 / 5
 ... vs tyr | score=1 | aver.=1.10682 | matched atoms=4 / 4
 Scoring is done.
```

with the most likely candidates as phenylalanine and cysteine. Match against these two residues:

```
Matching pattern pa5 versus phe
            9.274   5.022   3.288   2.526
          H H H H
 HN         1.018  -4.298  -6.465  -7.418
 HA         9.550   0.692  -2.921  -4.508
 HB1       23.050   7.864   1.671  -1.050
 HB2       21.764   6.579   0.386  -2.336
 HD1        7.867  -7.881 -14.304 -17.126
 HE1        6.813  -7.360 -13.140 -15.680
 HZ         7.117  -7.545 -13.524 -16.152
 HE2        6.813  -7.360 -13.140 -15.680
 HD2        7.867  -7.881 -14.304 -17.126
 HD*        7.867  -7.881 -14.304 -17.126
 HE*        6.813  -7.360 -13.140 -15.680

 Matching pattern pa5 versus cys
            9.274   5.022   3.288   2.526
```

```
          H H H H
HN          1.391  -4.683  -7.160  -8.249
HA          6.232   0.563  -1.749  -2.765
HB1        16.879   5.689   1.126  -0.879
HB2        16.089   4.900   0.337  -1.668
```

Since we do not know at this stage what the residue type is, we can leave that undetermined and let the automated routines come up with a possible answer later.

27. Finding the sequential connectivities

Once a set of patterns is determined, the next step is to connect these patterns. This is possible with the neighbor-finding algorithm. Generally, it is very important that your spectrum-specific shifts for the NOE spectrum be set for all patterns, as well as for the root frequencies, before you attempt to perform this action.. In this tutorial, however, proceed without doing that for each pattern. Also, make sure to select the NOE spectrum from the ***Experiments*** *table.*

If NOESY is not displayed, select the NOE spectrum from the Experiments table and click the **Draw** icon.

Select the **Assign/Neighbor/Find Neighbor Via 2D NOE** menu item. Set these values:

Experiment	**noe**
Root Frequency Tolerance	**0.015**
Frequency Collapse Tolerance	**0.015**
Output Level	**Low**

Leave the other parameters at their default values and click **More...** to see the default parameters.

In the next control panel, leave the defaults as they are and click **OK**.

After one or two seconds the results are printed and stored:

```
Spectrum   :(zn.mat)
 Tolerances :(   0.010    0.010  )
Root & Collapse tolerances:      0.0100000        0.0150000
 Pattern  :(ALL)
 Now at pattern :(          1  )
```

```
 Use :(   5.369    1.786    0.885  )
 Neighbors :(       2  )
 Scores    :(   1.000  )
 Now at pattern :(          2  )
 Use :(   3.060    2.845    2.665  )
 Neighbors :(       3  )
 Scores    :(   1.000  )
 Now at pattern :(          3  )
 Use :(   3.972    1.983    1.498    1.674  )
 Neighbors :(       4  )
 Scores    :(   1.000  )
 Now at pattern :(          4  )
 Use :(   4.049    2.545    1.366    0.749  )
 Neighbors :(       5        3  )
 Scores    :(   0.600    0.400  )
 Now at pattern :(          5  )
 Use :(   5.022    3.288    2.526  )
No candidate neighbors
```

From this listing you can see which pattern is neighbor to which, i.e., what the sequential connection is (i - i+1). For example, pa2 is neighbor to pa1, pa3 is neighbor to pa2, pa4 is neighbor to pa3, and pa5 is neighbor to pa4.

You can also check the stored values by selecting the **Assign/Neighbor/List Neighbor** menu item (or by using the **Spinsystem** table's **Spinsystem/List i+1 Neighbors** control, having selected the first pattern from the table).

Click **pa1** and leave **i - i+1** for **Order**.

Click **OK**.

In the output window you see:

```
The possible neighbors for pattern pa1 are:
pattern pa2 with probability: 1.0000
```

28. Visually verifying the results of neighbor detection

First click the third row (**pa3**) in the **Spinsystems** table, then <Ctrl>-click to select the fourth row (**pa4**).

Click the **Tile Plot** icon.

You now should see the results as a tile plot of the inter-pattern peaks.

Select **Contour** from the popup in the main icon bar to go to the contour plot.

Go to the **Spinsystems** table and click the **Draw** icon to overlay frequencies on the plot.

Now you see the frequencies displayed on top of the tile plot.

Inspecting the plot reveals that there are really inter-residue (inter-pattern) cross peaks. There is a well-defined cross peak at frequencies 8.928 and 9.094 ppm, which is an amide–amide cross peak between the two neighboring residues ($dH_{N(LYS)}H_{N(ILE)}$). Also, there is a cross peak between 3.972 and 9.094 ppm, which is an alpha–amide cross peak ($dH_{\alpha(LYS)}H_{N(ILE)}$). There is a cross peak at 1.893 and 9.094 ppm, which can be a beta–amide cross peak, since from residue matching you can see that this frequency likely belongs to a beta proton in the lysine residue. These two (three) interactions usually determine a sequential connectivity.

After neighbor detection, the next step is to match the found patterns against the known amino acid sequence.

29. Matching the found patterns against the known amino acid sequence

Select the **Assign/Sequential / Systematic Search** menu item. Leave the settings in the control panel at their defaults, except for the following:

Last residue (#) to consider53
Max # of assignments to generate100
Output level Medium

Click **OK**.

After a few seconds, the suggestion is ready. The output contains information about several steps in the automated routine:

```
   Constructing assignment-probability matrix
Probs for ALA :( 0.000 0.000 0.000 0.000 0.000 )
Probs for LYS :( 0.990 0.000 0.800 0.000 0.000 )
Probs for TRP :( 0.000 0.000 0.000 0.000 0.000 )
Probs for VAL :( 0.990 0.000 0.000 0.000 0.000 )
Probs for CYS :( 0.000 0.990 0.000 0.000 0.990 )
Probs for LYS :( 0.990 0.000 0.800 0.000 0.000 )
Probs for ILE :( 0.990 0.000 0.000 0.990 0.000 )
Probs for CYS :( 0.000 0.990 0.000 0.000 0.990 )
Probs for GLY :( 0.000 0.000 0.000 0.000 0.000 )
...

Constructing neighbour-probability matrix
Nbrs for null :( 0.000 1.000 0.000 0.000 0.000 )
Nbrs for null :( 0.000 0.000 1.000 0.000 0.000 )
Nbrs for null :( 0.000 0.000 0.000 1.000 0.000 )
Nbrs for null :( 0.000 0.000 0.400 0.000 0.600 )
Nbrs for null :( 0.000 0.000 0.000 0.000 0.000 )

Generating the assignments ...
... found 0 stretches starting at residue 1
... found 0 stretches starting at residue 2
... found 0 stretches starting at residue 3
... found 1 stretches starting at residue 4
... found 1 stretches starting at residue 5
... found 0 stretches starting at residue 6

   ...
```

```
   Number of assignments generated :( 2)
Buffer usage pointers (%) :( 0.040 )
Buffer usage assignments (%) :( 0.002 )
Sorting out the generated assignments
Assignments left :( 1 )
assignment # 1 -- length = 5 residues
...stretch of residues = 4 - 8 total scores:4.76 3.60
Residues:VAL_4 CYSH_5 LYS+_6 ILE_7 CYSH_8
Patterns: 1            2       3       4              5
Scores:         0.99    0.99    0.80    0.99    0.99
I>I+1:          1.00    1.00    1.00    0.60
The Pattern Suggest Assignment took 1 seconds!
```

The program thus suggests that pa1 belongs to residue 4 (VAL_4), pa2 belongs to residue 5 (CYSH_5), pa3 belongs to LYS+_6, pa4 belongs to ILE_7, and pa5 belongs to CYSH_8. The residue type of this latter spin system was in question—based on frequencies, the program could not distinguish between cysteine and phenylalanine. Now this ambiguity is resolved through the use of systematic search.

A new spreadsheet came up—one which contains this possible sequential assignment in tabular form: ***Stretches****. Now you can make sequence-specific assignments for the known frequencies.*

30. Making the sequence-specific assignment for pa1

Now you have two options:

a. Use the ***Stretches*** *table to make a quick assignment, then recheck the results and possibly edit the assignments using the* ***Spinsystems*** *table.*

b. Use the following sequence of commands:

Select the **Assign/Assign Spin System/Frequency** menu item. Select **pa1** from the list of patterns and click **Next**.

Following the procedures in Step 21, you next assign the sequence-specific assignment for each frequency of pa1.

Click **9.703** ppm in **Frequency list** and then click **Select**.

Now select VAL from the Residues list. Click **Filter**.

Next select **4** from the **#'s** list, and **HN** from the **Protons** list. Click **Build**.

Select **1:VAL_4:HN** from the **Possible Assignment** list.

Click **Add**. Now select the next frequency at 5.369 ppm, and use the same procedure to reassign this to **1:VAL_4:HA**.

*Alternatively, you can select any item in the **Assignments** list and then edit it in the **Atom Spec** box. Next, click ADD to accept it.*

*You can do the assignments on the **Spinsystems** table, too. For this, you just edit the fields next to the resonances.*

After finishing the last frequency, click **New Pattern** and make sequence-specific assignments for each pattern.

Once you assign the frequencies, you must transfer these assignments to peaks in order to use them together with volume measurements of those peaks in a refinement procedure.

31. Checking the NOE peaks

Make sure that the NOE spectrum is active. Display the full spectrum by pressing <Ctrl>-f while in intensity mode.

Displaying a full-spectrum contour plot with several contour levels can be time consuming. Using the hot keys <Ctrl>-i is an alternative.

Select the **Preference/Peak Display** menu item to see whether any peaks are assigned.

In the first control panel, set the **Coloring Mode** parameter to **By Assignment** and click **Draw**.

In the following control panel, set these values:

Fully assigned	**Green**
Partially (D1) assigned	**Magenta**
Partially (D2) assigned	**Cyan**
Multiply assigned	**Blue**
Not assigned	**Red**

Click **OK**.

All the peaks should now be red, indicating that none of them are assigned yet, although some of the frequencies are assigned.

Now you need to transfer frequency assignments to peak assignments.

32. Automatically generating peak assignments from frequency assignments

Select the **Assign/Peak Assign/Autoassign Peaks** menu item and set these values:

Rejection Cutoff	**8**
Output Level	**Quiet**
Unambiguous Cutoff	**6.0**
Strictly Enforce Distance	**Yes**
Peak Entity	**xpk:noe.**
Multiple Assign	**off**
Skip Fully Assigned	**off**
Skip Multiple Assigned	**off**

Click **OK**.

In a few seconds you should see output similar to the following:

```
   Assign peaks for spectrum :(noe)
Tolerances :( 0.010 0.010 )
Spins (h h)
Folding (0 0 )
Transfers ( N )
Nr of peaks unambiguously assigned :( 95 )
Nr of peaks with competing assmnts :( 0 )
Nr with no or too many assignments :( 1787 )
The peak auto assignment took 9 seconds
```

The cross peaks have different colors, depending on the assignment status: green for fully assigned peaks, red for non-assigned peaks, blue for multiply assigned peaks, and turquoise and purple for partially assigned peaks. You should see several green peaks, with the majority of peaks still being red.

Next you go back and check whether the peaks belonging to different patterns were assigned correctly.

33. Checking the peak assignment for pattern 1

Go to the **Spinsystems** table and select the first pattern (**pa1**). Then click the **Tile Plot** icon.

Now draw the peaks if they are not drawn, using the **View/ Draw Peaks** menu item. Notice that the different peaks are in different colors, depending on assignment status.

The peak at 9.698 and 5.370 ppm is now green, showing that the peak was assigned along both frequencies. The peak at 5.37 and 9.64 ppm and the symmetric peak at 9.64 and 5.37 ppm are both red, showing that the peaks have not been assigned yet.

34. Checking the inter-residue peak assignment

Next you follow a similar procedure for inter-residue peaks.

Go to the **Spinsystems** table and select the first pattern by clicking its row. Then <Ctrl>-click to select the second pattern (**pa2**).

Click the **Tile Plot** icon.

Press <Ctrl>-k if peaks are not drawn.

The peak at 5.369 and 9.194 ppm is green, denoting that this is a fully assigned inter-residue peak between VAL_4 and CYSH_5.

Now you can list the peak.

Select the **Assign/List Peak** menu item and, with the resulting cross-hair cursor, pick the peak at 5.369 and 9.189 ppm.

Click the <Esc> key to exit the peak listing mode.

You now should see output in the output window that looks like:

```
Peak # 133 Intensity = 0.336250e+06
 Dimension Position (ppm) Width (Hz)       Peak Assignment
 W2      5.36633          20.95018         1:VAL_4:HA
W1       9.18967          17.5624          1:CYSH_5:HN
 Dimension       Frequency Assignment      Distance(ppm)    Pattern id
W2       1:VAL_4:HA               0.003056         pa1
W1       1:CYSH_5:HN              00.0065804       pa2
```

*This indicates an $d_{\alpha N}(i,i+1)$ NOE connectivity. If you have the corresponding peak table open as a spreadsheet (**Peaks-xpk:noe**), this peak is highlighted in the table.*

*Note the red peak at around 9.7 and 9.1 ppm, which is in the lower-left box of the tile display—if you list it with the **Assign/List Peak** menu*

item, you see that the two frequencies defining this peak are assigned to 1:VAL_4:HN and 1:ILE_7:HN but that the peak itself was not assigned:

```
Peak # 154          Intensity = 0.464310e+05
Dimension     Position (ppm)      Width (Hz)    Peak Assignment
W2             9.70856    21.65628
W1             9.08714    20.65723

Dimension  Frequency Assignment Distance(ppm)  Pattern id
W2           1:VAL_4:HN   0.0030603        pa1
W1           1:ILE_7:HN   0.0090561        pa4
```

This is because the two atoms are farther apart than the NOE cutoff used in automated assignment (8 Å). You can check this with the following action.

Select the **Measure/Distance/Separation** menu item or click the **Measure Distance** icon. Click this peak.

You see the following output in the output window:

```
Peak # 154

Frequency Assignment:

   W2                W1                      Distance (A)
 1:VAL_4:HN          1:ILE_7:HN                    11.1772
```

This proves that the peaks were not assigned because the distance criterion was not met.

*Turn off the tile mode using the **View/Tile Plot/Tile Plot** menu item.*

To generate structures you need to assign all the peaks. Usually the peak assignment should be done on an NOE spectrum where buildup (i.e., multiple mixing time experiments) information is also available. There is a spectrum—a 450-ms mixing-time NOESY experiment which is defined in the following database.

35. Reading in the database containing fully-assigned patterns

Select the **File/Open** menu item. Select **DBA** for the **File Type** and select the **zn_model.dba** file from the list. Click **OK**.

If it prompts you to save changes to the previous DBA file you opened, click **Save Changes** to save them.

All tables are automatically closed. Select **Assign/ Experiment** to open the Experiments table. Click **OK** to the next dialog box. Select **Edit/Spin Systems** to open the Spinsystems table.

Now select the **buildup** experiment using the **Experiments** table, highlighting the fourth row and clicking the **Draw** icon. Click the **Full Plot** icon to draw the full plot if necessary.

The spectrum-specific shifts for this experiment are not exactly set yet, you need to adjust them in the next step:

Select the **Assign/Spin System/Auto Update Specific Shifts** menu item. Check **Use All Patterns**. Set the **Spectrum Specific Shift** parameter to the **buildup** experiment. The **D1** and **D2 Tolerance** should be set to **0.015**. Click **OK**.

The next step is to assign all the peaks with the help of these newly defined spectrum-specific chemical shifts.

36. Assigning the buildup automatically

Select the **Assign/Peak Assign/Autoassign Peaks** menu item and be sure that these values are set:

Rejection Cutoff	**8.0**
Unambiguous Cutoff	**6.0**
Strictly Enforce Dist	**on**
Peak Entity	**xpk:buildup**
Multiple Assign	**off**
Skip Fully Assigned	**off**
Skip Multiply Assigned	**off**
Output Level	**Quiet**

Click **OK**.

After one or two minutes the auto-assignment is done. You should see something like this in the output window:

```
   Generating automatic assignments
      Please wait ...
Press <Esc> to quit.
Assign peaks for spectrum :(buildup)
                              W1     W2
  Spins                      :(hh )
  Folding                    :(        0         0   )
  Transfers                  :(N )
  Tolerances                 :(   0.010     0.010   )
  Nr of peaks unambiguously assigned :( 858)
  Nr of peaks with competing assmnts :(        0   )
  Nr of peaks with no new assignment :( 1104 )

The peak auto assignment took 81 seconds
```

The following step is to define NOE distance restraints from this spectrum. In restraint definition, the first step is to define a scalar peak, for which the distance between the atoms it is assigned to is fixed. There are several ways of doing this, but for now we will demonstrate with a fixed HB1–HB2 peak.

37. Defining the scalar peak

Select the **Peaks/Find** menu item. Set these values:

Find Peak	**By Number**
Action	**Zoom+Color**
Peak ID	**1153**

Click **OK**.

*List this peak with the **Assign/List Peak** menu item:*

```
Peak # 1153          Intensity = 0.2238639e+07
Dimension      Position (ppm)      Width (Hz)     Peak Assignment
F2             2.255588            32.96875         1:ASP-_13:HB1
F1             2.859035            36.89453         1:ASP-_13:HB2
Dimension   Frequency Assignment        Distance(ppm)       Pattern id
F1             1:ASP-_13:HB2          0.001035             pa6
F2             1:ASP-_13:HB1          0.4119873e-03        pa6
F1             1:ASP-_35:HB2          0.96488e-03         pa12
```

This will be the scalar peak.

Select the **Measure/Scalar_Normalize** menu item. In the control panel, set the **Add One** option and click **OK**.

In the next control panel, set the values:

Peak Name D1	**1:ASP-_13:HB1**
Peak Name D2	**1:ASP-_13:HB2**
Distance	**1.75**

Click **OK**.

*Now set the intensity plot (if you were in contour plot) and draw a full plot (**View/Limits/Full Limits**).*

38. Defining the restraints

Select the **Measure/DISCOVER Restraints** menu item. In the first control panel, set **Restraint Class** to **NOE Distance** and **Action** to **Define**, then click **OK**.

Set these values in the next control panel:

Action	**New**
Scalars	**etc:scalars**
Lower Force Const	**30.0**
Upper Force Const	**30.0**
Maximum Force	**1000.0**
Calculation Method	**Single tm**
Mixing Time	**0.1**
Symmetry Selection	**Use Weaker**
Method	**S-M-W Bins**
Partial Overlap	**Use as Qual**
Are Threshold	**50**

Click **OK**.

In the third control panel, set these values:

Strong	**-1.0 2.5**
Medium	**-1.0 3.5**
Weak	**-1.0 6.0**

Click **OK**.

In the fourth control panel, leave **Lower Bound** at **-1.0** and **Upper Bound** at **6.0** for the overlapped peaks. Click **OK**.

Yellow footprints appear on the plot, indicating the peaks from which restraints are generated. At the end of the procedure a message appears:

```
474 NOE distance restraints calculated
From these 51 were qualitative distance restraints because of partial overlap
0 peaks were discarded because of assignment problems
```

*After you have finished peak assignment and restraint generation, you can move on to generate structures in **NMR Refine**. Therefore, the last step is to write out a database that you can import to Insight II.*

39. Writing the database

Select the **File/Export/Restraints** menu item. Set **Peaks and Resonances** and **Restraints** to **on**.

For all the filenames (**Peak Intensity File**, **Chemical Shift File**, **Assignment File**, **Restraint File**) enter: **znrdlec** and set the **Type** to **DISCOVER.**

Click **OK**.

This action writes out the znrdlec.pks, znrdlec.ppm, znrdlec.asn, and znrdlec.rstrnt files.

After running DGII or simulated annealing, the first structures are generated. A new set of assignments can be generated based on this new structure(s). First you can redefine the molecular structure and then rerun auto-assignment.

40. Redefining the coordinates

Select the **Assign/Read Coordinates** menu item. From the list select **znrddg.car** and click **OK**.

This replaces the linear-chain coordinates of Zn–rubredoxin with the first DG-II structure coordinates.

41. Rerunning autoassignment for the buildup

First unassign the peaks by selecting **Assign/Peak Assign/ Unassign Peaks** and leave the peak entity as **xpk:buildup**. Click **OK**.

Select the **Assign/Peak Assign/Autoassign Peaks** menu item and set these values:

Rejection Cutoff	**8.0**
Strictly Enforce Dist	**on**
Unambiguous cutoff	**6.0**
Peak Entity	**xpk:buildup**
Multiple Assign	**off**
Skip Fully Assigned	**off**
Skip Multiply Assigned	**off**
Output Level	**Quiet**

Click **OK**.

In a few minutes you see:

```
Generating automatic assignments
  Please wait ...
  Press <Esc> to quit.
Assign peaks for spectrum :(buildup)
                             W1     W2
Spins                       :(hh )
Folding                     :(        0         0   )
Transfers                   :(N )
Tolerances                  :(   0.010     0.010   )
Nr of peaks unambiguously assigned :(    1123 )
Nr of peaks with competing assmnts :(       0   )
Nr of peaks with no new assignment :(      839 )

The peak auto assignment took 87 seconds
```

More than 250 new peak assignments were made based on the preliminary DG-II structure.

42. Regenerating the restraints

Select the **Measure/DISCOVER Restraints** menu item. In the first control panel, set **Restraint Class** to **NOE Distance** and **Action** to **Define**. Click **OK**.

Set these values in the second control panel:

Action	**New**
Scalars	**etc:scalars**
Lower Force Const	**30.0**
Upper Force Const	**30.0**
Maximum Force	**1000.0**
Calculation Method	**Single tm**
Mixing Time	**0.1**
Symmetry Selection	**Use Weaker**
Method	**S-M-W Bins**
Partial Overlap	**Use as Qual**
Are Threshold	**50**

Click **OK**.

In the third control panel, set these values:

Strong	**-1.0 2.5**
Medium	**-1.0 3.5**
Weak	**-1.0 6.0**

Click OK

And in the fourth control panel, leave **Lower Bound** at **-1.0** and **Upper Bound** at **6.0** for the overlapped peaks. Click **OK**.

Yellow footprints again appear on the plot, indicating the peaks from which restraints are generated. At the end of the procedure a message appears:

```
633 NOE distance restraints calculated
From these 61 were qualitiative distance restraints because of partial overlap
0 peaks were discarded because of assignment problems
```

43. Redefining restraints

Typically, after a DGII or simulated annealing run you need to analyze your restraints. This can be done in Insight II, and the results can be printed as a file containing a list of restraints that are violated in multiple structures. In FELIX you can then use that file to help you to redefine or reassign erroneous assignments or restraints. This is what you do in the next step.

Select the **Measure/DISCOVER Restraints** menu item again. In the control panel set **Restraint Class** to **NOE Distance** and **Action** to **Redefine**.

Click **OK**.

In the second control panel, enter **zn_viol01.txt as** the **Filename** as and leave the other parameters at their defaults. Click **OK**.

*The program now brings up a new spreadsheet containing the distance restraint violations—**Violations**. In this table you can zoom in on the peak defining the first problematic restraint and can also see the values of the restraint and the violations.*

*Select the first row in the **Violations** table and click the **Zoom** icon. The restraint for 1:GLU-_47HN and 1:GLU-_47:HG2 which had a restraint between 1.8 and 4.5 Å was violated in 14 conformations out of a total of 20, and the violation average was 0.17 Å. The average distance measured in the 20 conformation is 4.64 Å and the calculated distance based on ISPA is 2.78 Å. You can see that this peak is heavily overlapped, and the symmetric peak has not been assigned at all (Click the*

Symmetric Peak *icon to check). Since this restraint is very unreliable, you may want to delete it.*

Go to the **Violations** table and select the **Violation/Delete Restraint** menu item.

The output window now says:

```
Item 320 deleted from biosym:noe_dist.
```

Now you can see in the ***NOE-Restraints*** *table that this restraint was indeed deleted from the database.*

Now select the second violation from the **Violations** table and click the **Zoom** icon.

A new peak appears in the spectrum, which is the next problematic restraint. This is a well-defined peak and the distance calculated on the symmetric peak is larger than the one from this peak (use ***Violation/ Calculate Distance*** *in the* ***Violations*** *table) and also larger than the original restraint was:*

```
Peak 1043  1:GLY_42:HN - 1:CYSH_38:HB2  distance: 3.202873
Symmetric peak 369  distance: 3.564213
```

You may want to simply increase the bounds for this as in the following step.

Select the **Violation/Redefine Bounds** menu item from the **Violations** table. Set **Lower Bound** to **-1.0** and **Upper Bound** and **Upper Bound with Correction** to **5.0**. Click **OK**.

The program now informs you:

```
Item 243 updated in biosym:noe_dist.
```

Since there is no other violated restraint left in this file, this finishes the redefinition.

44. Calculating the chemical shift index

The chemical shifts of certain spins can be informative about regular secondary structural elements. This can be exploited as shown in the following step.

Since this is homonuclear data, select the **Assign/Chemical Shift Index/HA Chemical Shift Index** menu item. Set these values:

Library Name	**csi.rdb**
CSI File	**zn:hacsi**
File to Export	**csi.txt**
Molecule Name	**ZNRDDG**

Click **OK**.

*In few moments the calculation is done and a spreadsheet appears (**HA-CSI**) showing the residues, the assigned HA chemical shifts, and the CSI index and grouping, as well as the Richardson classification. By browsing through the table you can see regions that were found to be beta-sheets or alpha-helices. The program also wrote a file csi.txt with this classification to the disk. This file can be imported into Insight II and can (for example) be rendered on the ZNRDDG molecule.*

45. Writing the database

Select the **File/Export/Restraints** menu item. Set **Peaks and Resonances** and **Restraints** to **on**.

For all the filenames (**Peak Intensity File**, **Chemical Shift File**, **Assignment File**, **Restraint File**) enter: **znrddg**, set the **Type** to **DISCOVER**, and click **OK**.

This action writes out the znrddg.pks, znrddg.ppm, znrddg.asn, and znrddg.rstrnt files.

46. Exiting FELIX

To exit FELIX, select the **File/Exit** menu item.

Lesson 2: Heteronuclear double-resonance 3D assignment strategy

This tutorial shows typical steps involved in assignment of a singly-labelled protein. The data set is the ^{15}N-HSQC, ^{15}N-HMQC-TOCSY, and ^{15}N-HMQC-NOE spectra of the ^{15}N-enriched MCP-1 protein from P. J. Domaille (DuPont Merck, Wilmington) and T. Handel (University of California, Berkeley).

The topics covered in this lesson are:

- Database set up.
- Spin-system detection using double-resonance spectra.
- Spin-system identification.
- Spin-system neighbor detection.
- Sequence-specific assignment of spin systems using systematic search.
- Sequence-specific assignment of spin systems using *simulated annealing.*

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in “Setting up tutorial files” in the preface, *How To Use This Book.*

The files for this lesson are all located in the **Assignment\Lesson2** folder

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**. If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, Navigate to your working directory. If you used the default suggestion, this will be: **C:\Felix_Practice\Assignment\Lesson2**.

Enter mcp.dba and select **OK** to build a new database file.

3. Setting up the database

Select the **Assign/Project** menu item.

FELIX informs you that no project was found in the database.

Click **OK** to acknowledge that you want to build a new project.

FELIX then asks you for a new project name.

The default name in this new dialog box is **asg:project**. Enter a different name if you want (for example, **mcp:project**)

In the second control panel, set this value:

File name	**mcp1_lec.car**
File type	**Insight Molecule (*.car)**

Click **OK** to build the entities and read in the molecule.

After this step is successfully completed, a library should be defined. The library is an ASCII file, as described in the Assign/Define Library section of Chapter 5, Assign User Interface, in the FELIX User Guide. FELIX contains a standard library (pd.rdb) which you should read in.

In the third control panel, toggle the **Define Library From File** parameter **on** and click **OK**.

In the fourth control panel, select **pd.rdb** and click **OK**.

4. Adding experiments to the projects

Select the **Assign/Experiment** menu item to define new experiments in the assignment database. When the list of names of matrices appears, select **hsqc.mat** (the ^{15}N-HSQC spectrum).

Click **OK**.

Set these values for the plot, in the **2D Display Parameters** control panel:

Contour Threshold	**0.02**
Color Scheme	**Blue/Green**
Axis type D1	**ppm**
Axis type D2	**ppm**
D1 Scale	**1**
D2 Scale	**2**

Leave the other parameters at their default values and select **Apply**.

Click **OK** when the message box appears.

If you want, you can change the display parameters by going to the ***Experiments*** *table and using its* ***Experiment/Change Attribute*** *menu item later.*

The program plots a density or contour plot of the ^{15}*N-HSQC spectrum using the parameters you defined.*

Now another control panel appears.

Set the parameters to these values:

Title	**hsqc**
Use Default Names	**on**
Type	**2D N-15 HSQC**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Proton**
D2 Nucleus	**Nitrogen**
D1 Tol	**0.02**
D2 Tol	**0.07**
D1 Fold	**No**
D2 Fold	**No**
W1	**D2**
W2	**D1**
W1-W2 Transfer	**J-coupled**
# of J Steps	**1**

Click **OK**.

The spectrum-specific tolerances are important to define and are used in many automated and semi-automated procedures.

A new window containing an Experiments table is open and displayed to the left of the spectral window. Note that, by default, whenever a new window (table or spectral) is open, FELIX automatically re-arranges the layout of the windows. You can turn off this feature by selecting ***Preference/Frame Layout*** *from the main menu and set* ***Action*** *to* ***None****. You can also do the automatic re-arrangement at anytime by selecting* ***Window/Auto Arrange****.*

Note: When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

5. Adding the ^{15}N-HMQC-TOCSY experiment to the projects

Activate the **Experiments** table so that its own menu is displayed. Select the **Experiment/Add** menu item to define the next experiment in the assignment database. When the list of names of matrices appears, select **mcpn15tocsy.mat** (the ^{15}N-HMQC-TOCSY spectrum).

Click **OK**.

Set these values for the plot using the **3D Display Parameters** control panel:

Contour Threshold	**0.5e-5**
Color Scheme	**Magenta**
Number Of levels	**16**
Negative Levels	**Off**
Axis type D1	**ppm**
Axis type D2	**ppm**
Axis type D3	**ppm**
D1 Scale	**1**
D2 Scale	**1**
D3 Scale	**1**

Leave the other parameters at their default values and select **Apply**.

Click **OK** when the message box appears.

*If you want, you can change the display parameters using the **Experiment/Change Attribute** menu item in the **Experiments** table later.*

The program plots a density plot or contour plot of the first D1–D2 plane of the ^{15}N-HMQC-TOCSY spectrum using the parameters you defined.

Now another control panel appears.

Set the parameters to these values:

Title	**tocsy**
Use Default Names	**on**
Type	**3D HSQC-TOCSY**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Proton**
D2 Nucleus	**Proton**
D3 Nucleus	**Nitrogen**
D1 Tol	**0.02**
D2 Tol	**0.04**
D3 Tol	**0.1**
D1 Fold	**No**
D2 Fold	**No**
D3 Fold	**No**
W1	**D3**
W2	**D1**
W3	**D2**
W1-W2 Transfer	**J-coupled**
# of J Steps	**1**
W2-W3 Transfer	**J-coupled**
# of J Steps	**7**

Click **OK**.

6. Repeating Step 4 for the ^{15}N-HMQC-NOE spectrum

Again activate the Experiments Table and select the **Experiment/Add** menu item.

Set these values:

15N-HMQC-NOE	**mcpn15noe.mat**
plotting parameters:	
Contour Threshold	**0.2e-4**
Number Of Levels	**16**
Level Multiplier	**default setting**
Color Scheme	**Cyan**
Axis Type	**ppm**
Negative Levels	**Off**

Click **OK**.

In the next control panel select these values:

Experiment Title	**noe**
Use Default Names	**on**
Type	**3D HSQC-NOESY**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Proton**
D2 Nucleus	**Proton**
D3 Nucleus	**Nitrogen**
W1	**D3**
W2	**D1**
W3	**D2**
W1-W2 Transfer	**J-coupled**
W2-W3 Transfer	**NOE**
# of J Steps	**1**
Mixing time	**0.1**
spectrum specific tolerances	
D1	**0.02**
D2	**0.025**
D3	**0.1**

Click **OK**.

7. Reading in the peaks

Select the first row in the **Experiment** table by clicking it to chose the **hsqc** experiment. Then click the **Draw** icon.

Activate the spectral window so that the main menu is displayed. Select the **File/Import/Peaks** menu item. Set the **Selection** parameter to **hsqc.xpk**. Leave the **FELIX Peak Table Name** parameter at its current value (**xpk:hsqc**) and the **Peak File Type** as **FELIX Peak File**. Click **OK**.

When the program asks you whether to overwrite the entity, click **OK**.

This command reads in the peaks and displays them in a spreadsheet. The peaks are also displayed as boxes.

Activate the Peaks table and select **File/Close** to close it.

Select the second row in the **Experiments** table and click the **Draw** icon to display the TOCSY spectrum.

Activate the spectral window and select the **File/Import/Peaks** menu item. Set the **Selection** parameter to **mcpn15tocsy.xpk** and click **OK**.

When the program asks you whether to overwrite the entity, click **OK**.

Close the Peaks-xpk:tocsy table by selecting **File/Close** from its menu or by clicking the **X** button.

Select the third row in the **Experiments** table and click the **Draw** icon to display the **noe** spectrum.

Activate the spectral window and select the **File/Import/Peaks** menu item. Set the **Selection** parameter to **mcpn15noe.xpk** and click **OK**.

When the program asks you whether to overwrite the entity, click **OK**.

Close the Peaks-xpk:noe table.

Now you have a full peak set defined for all three experiments.

8. Selecting the HSQC spectrum

Select the HSQC spectrum in the **Experiments** table and click the Draw icon.

Activate the spectral window. Press <Ctrl>-f to obtain the full plot if necessary.

*The next step is the collection of prototype patterns, that is, sets of frequencies, which are later promoted to patterns and assigned to specific amino acid residues. The commands connected to prototype patterns are in the third subsection of the **Assign** pulldown. Since we have the ^{15}N-HSQC, ^{15}N-HMQC-TOCSY, and the ^{15}N-HMQC-NOESY spectra in our project, we demonstrate the use of the two currently available double-resonance prototype pattern-collection methods.*

9. Performing prototype pattern detection

Select the **Assign/Collect Prototype Patterns** menu item. In the control panel, select **Double Resonance** for **Method** and click **OK**.

In the second control panel, set the **Method** to **3D HS(M)QC-TOCSY** and click **OK**.

You see a control panel with several options. The program tries to automatically fill in reasonable values

Make sure these values are set in the third control panel:

Experiment	**tocsy**
Root (Amide Proton) Dimension	**D1**
Frequency Collapse Tolerance	**0.08**
Output Level	**Low**

Now click **More...** and set these values:

Seed Area D1 Low:	**5.2**
High:	**12.0**
Seed Area D2 Low:	**5.2**
High:	**12.0**
Seed Area D3 Low:	**90**
High:	**130.0**
Use Seed Peak D1	**on**
D2	**off**
D3	**on**
Exp. Area D1 Low:	**5.2**
High:	**12.0**
Exp. Area D2 Low:	**-2.0**
High:	**12.0**
Exp. Area D3 Low:	**90.0**
High:	**130.0**
Use Exp. Peak D1	**off**
D2	**on**
D3	**off**
Remove Intraproto Frequencies	**on**
Number of Frequencies in Proto Min	**3**
Max	**12**
Number of Iterations	**8**
Frequencies per Iteration	**1**

Leave the other parameters at their defaults and click **OK**.

Information about the current stage of prototype pattern collection appears in the output window. After one or two minutes, the prototype pattern collection finishes and a spreadsheet of prototype patterns is displayed. The following information appears in the output window:

```
   Nr of protos generated : ( 52)
The 3D protopattern detection took 4 seconds
```

The protein has 77 residues, from which you can theoretically expect only 71 spin systems to be found, since there are 5 prolines and the N-terminal spin system is probably missing. If you have recorded a well resolved 2D ^{15}N-HSQC spectrum, then that can greatly help in spin-system collection. Therefore, we present here the other prototype pattern-detection method implemented in FELIX.

10. Performing the second prototype pattern detection

Select the **Assign/Collect Prototype Patterns** menu item. In the control panel, select the **Double Resonance** option and click **OK**.

In the second control panel, select the **2D HSQC + 3D HS(M)QC-TOCSY** option and click **OK**.

You get a third control panel with several options. The program tries to automatically fill in reasonable values.

Set these values in the third control panel:

Seed Experiment	**hsqc**
Root Dimension	**D1**
Expansion Experiment	**tocsy**
Seed/Expansion Region	**Use**
Defaults	
Frequency Collapse Tolerance	**0.08**
Output Level	**Low**

Now click **More...** and set these values in the resulting control panel:

Seed Area D1 Low:	**5.2**
High:	**12.0**
Seed Area D2 Low:	**90.0**
High:	**130.0**
Use seed peak D1	**on**
D2:	**on**
Expansion Area D1 Low:	**5.2**
High:	**12.0**
Exp. Area D2 Low:	**-2.0**
High:	**12.0**
Exp. Area D3 Low:	**90.0**
High:	**130.0**
Use Exp. Peak D1	**off**
D2	**on**
D3	**off**
Remove Intraproto Frequencies	**on**
Number of Frequencies in Proto Min	**3**
Max	**12**
Number of Iterations	**8**
Frequencies per Iteration	**1**

Leave the other parameters at their defaults and click **OK**.

Information about the current stage of prototype pattern collection appears in the output window. After one minute, the prototype pattern collection is finished, and the following information appears in the output window:

```
   Nr of protos generated : ( 13)
The 3D protopattern detection took 1 seconds
```

Now you have 65 prototype patterns in all. While this method relies on well resolved 2D HSQC peaks, the previous one depends on well resolved pseudo-diagonal peaks of the HMQC-TOCSY spectrum. In certain cases, the higher digital resolution and better-defined peak shapes of 2D spectra help find more spin systems, while in other cases relying on the third dimension yields better results. Sometimes the combination of the two is the best choice, as you can see from this example (the ^{15}N-HSQC + ^{15}N-HMQC-TOCSY would generate only 58 spin systems).

Since clearly some spin systems were missed, it is always advisable to inspect the peaks in the spectrum to see which ones were not assigned to spin systems. This procedure is shown in Step 13.

11. Visually inspecting several prototype patterns

Select the **tocsy** experiment using the **Experiment** table: click the second row and then click the **Draw** icon. Select the third row in the **Protopatterns** table. Click the **Zoom** icon in the table.

The region (strip) containing peaks of the 3rd spin system is displayed.

Now connect the HSQC and HMQC-TOCSY spectra.

12. Connecting the HSQC and TOCSY spectra

From the main menu select the **Window/Add New Window** menu item. Select **Tile** as the **Rearrange Layout** parameter.

Activate the new frame (Frame 2) if necessary. Select the **hsqc** experiment in the **Experiments** table and click the **Draw** icon to display it in Frame 2.

Select the **Preference/Frame Connection** menu item. In the control panel, set **First Frame** to **2** and **Second Frame** to **1**. Select **Custom** and click **OK**.

In the second control panel, set these values:

D1	**D1**
D2	**Null**
Define Jump	**on**
D2	**D3**
Null	**Null**

Click **OK**.

*This connects the D1(1H) of HSQC with D1(1H) of HMQC-TOCSY, and D2(15N) of HSQC with D3(15N) of HMQC-TOCSY. If you switch to the frame containing the **tocsy** experiment, you can zoom in on the spectra (**Zoom** in **Protopatterns** table), but this time display the same region in both spectra.*

You can use various methods to browse through the spectrum in Frame 2, and the same action occurs in Frame 1, too.

13. Coloring the peaks based on prototype patterns

Activate Frame 2 and click the **Full Plot** icon to draw a full plot.

Select the **Preference/Peak Display** menu item. Set **Coloring Mode** to **By Protos** and leave the other parameters at their defaults. Click **Draw**.

*A new control panel appears, where you can set the colors for peaks which have each frequency belonging to a prototype pattern (**To The Same**), and for those which do not (**None**).*

Leave the values in the second control panel at their defaults and click **OK**.

*From now on, when you use the **View/Draw Peaks** menu item, the peaks will be drawn according to this coloring scheme: green peak boxes will be drawn at peaks that belong to a prototype pattern, and red peak boxes will be drawn at peaks that were not assigned to any particular spin system. Therefore, the manual spin-system detection should proceed from those peaks which are, in this case, red.*

When the full plot is drawn you can see that there are red and green peak boxes. You may notice a red peak box at the lower edge of the HSQC spectrum at around 124 ppm and 8.7 ppm.

Click the **Zoom** icon and zoom in on that peak. Then move the crosshair cursor on the center of that peak and click it.

This moves the display of the 3D TOCSY spectrum at that particular plane.

Now you learn to create a new spin system manually.

Make sure Frame 2 is the current frame. Select the **Assign/Frequency Clipboard/Zero Clipboard** menu item to clear the clipboard in Frame 2.

Now you are ready to add frequencies to this clipboard.

Select the **Assign/Frequency Clipboard/Add One** menu item.

When the crosshair cursor appears, click with it on the peak at 8.74, 124.1 ppm.

In the control panel, set **Both 8.743666 124.1559** for **Frequency** and **HN** and **N** for **Nucleus 1** and **Nucleus 2** parameters, respectively and click **OK**.

Press the <Esc> key to quit the addition mode.

*You can check what is in the clipboard by listing it (**Assign/Frequency Clipboard/ View Clipboard**), and the result is printed in the output window:*

```
The Frequency Clipboard List contains the following
frequencies:

   #    Freq(ppm) Atom
  --- --------- ----
   1    8.744       H
   2   124.156      N
```

Now switch to the frame containing the 3D TOCSY spectrum.

Click anywhere in Frame 2 to activate it. Click the **Zoom** icon and zoom in to the two visible peaks.

Select the **Assign/Frequency Clipboard/Add One** menu item. With the crosshair cursor, click the peak at around 8.74, 5.18 ppm.

No peak box drawn for this peak, because that peak was missed during peak picking since it is on the very edge of the spectrum.

In the control panel, set **Frequency** to **F1_D2_H 5.182883** and **Nucleus 1** to **HX**. Click **OK**.

With the crosshair cursor, click the peak at around 8.74 and 3.03.

In the control panel, set **Frequency** to **F1_D2_H 3.031679** and **Nucleus 1** to **HX**. Click **OK**.

Since this is the last frequency, press the <Esc> key to quit.

*Since there were no peaks picked for these latter frequencies, your actual results may be different from the those presented. Check the clipboard again (**Assign/Frequency Clipboard/View Clipboard**):*

```
The Clipboard List contains the following frequencies:

   #   Freq(ppm) Atom

  --- --------- ----
   1    8.744       H
   2   124.156      N
   3   5.183        X
   4   3.032        X
```

14. Promoting the prototype patterns to patterns

Select the **Assign/Promote Prototype Patterns** menu item. In the first control panel, set the **Copy** parameter to **Prototype Patterns to Spin Systems (Patterns)** and click **OK**.

In the second control panel, do not change any values, just click **OK**.

*After couple of seconds, 65 new patterns are generated and displayed in a spreadsheet. You can inspect the patterns using the **Assign/Report Spin System** menu item:*

```
LISTING FOR PATTERN pa1

comment            :  fromproto1
color              :  Red
root frequency      :  8.451

             Frequencies
             -----------
 generic       specific       assignment
  shift                shifts
              1   2   3   4   5   6     7
     8     9
         hsqc tocsy noe null null null null null null
 8.451       8.451 8.451 8.451 8.451 8.451 8.451 8.451 8.451 8.451     1:*_*:HN
 123.444       123.444 123.444 123.444 123.444 123.444 123.444 123.444 123.444 123.444     1:*_*:N
 3.739       3.739 3.739 3.739 3.739 3.739 3.739 3.739 3.739 3.739     1:*_*:HX
 2.037       2.037 2.037 2.037 2.037 2.037 2.037 2.037 2.037 2.037     1:*_*:HX
 0.816       0.816 0.816 0.816 0.816 0.816 0.816 0.816 0.816 0.816     1:*_*:HX

       The neighbor probabilities
       --------------------------
     Pattern              Probability
       The residue type probabilities
     ------------------------------
      Residue              Probability

     (i-1) Frequencies from protopattern  1
   ----------------------------------------
```

15. Adding the manually detected spin system to the patterns

Select the **Assign/Frequency Clipboard/Copy Clipboard to Pattern** menu item. Keep the default parameters unchanged and click **OK**.

A new pattern with name pa66 is added to the database and is also displayed in the Spinsytsems table.

16. Scoring the patterns

Select the **Assign/Residue Type/Score Residue Type** menu item. Set these values:

Use All Patterns	**On**
Scoring Method	**All Atoms**
Min Atoms	**N**
Max Std Dev	**3**
Use COSY Peaks	**None**
Database	**Store**

Click **OK**.

*After a few minutes, all 66 patterns are scored and the residue type probabilities are stored. Using the **Assign/Report Spin System** menu item for the first pattern will give similar results:*

```
LISTING FOR PATTERN pa1

 comment            :  fromproto1
 color              :  Red
 root frequency     :  8.451

             Frequencies
             -----------
 generic       specific            assignment
  shift                shifts
              1     2     3     4     5     6     7     8     9
             hsqc tocsy noe null null null null null null
 8.451       8.451 8.451 8.451 8.451 8.451 8.451 8.451 8.451 8.451     1:*_*:HN
 123.444       123.444 123.444 123.444 123.444 123.444 123.444 123.444 123.444 123.444     1:*_*:N
 3.739       3.739 3.739 3.739 3.739 3.739 3.739 3.739 3.739 3.739     1:*_*:HX
 2.037       2.037 2.037 2.037 2.037 2.037 2.037 2.037 2.037 2.037     1:*_*:HX
 0.816       0.816 0.816 0.816 0.816 0.816 0.816 0.816 0.816 0.816     1:*_*:HX

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      ILE                 1.000
      VAL                 1.000
      THR                 0.833
      ARG                 1.000
```

```
   LYS                 1.000
   LEU                 1.000

(i-1) Frequencies from protopattern  1
-----------------------------------------
```

After the spin-system probabilities are defined, the next step is to find neighboring spin systems. This can be achieved here by using the ^{15}N-HMQC-TOCSY spectrum. In such a spectrum you can expect cross peaks between the spins of the i^{th} and $(i+1)^{th}$ residue, as well as between further separated residues. The algorithm should search for NOE cross peaks, such as $H_{N,i}$–$H_{N,i+1}$($-N_{i+1}$), $H_{\alpha,i}$–$H_{N,i+1}$($-N_{i+1}$), and $H_{\beta,i}$-$H_{N,i+1}$($-N_{i+1}$), whose presence makes the connectivity between the two spin systems probable. Before you start the neighbor search, the spectrum-specific shifts of the patterns for the ^{15}N-HMQC-NOESY spectrum should be updated.

17. Setting the spectrum-specific shifts for all patterns

Disconnect the frames by selecting the **Preference/Frame Connection** menu item and choosing the **Disable** option.

Select the NOE spectrum by selecting the **Experiment/ Select** menu item in the **Experiments** table.

Since the chemical shifts in the patterns were defined using the HSQC and the ^{15}N-HMQC-TOCSY spectrum, a slight difference is expected between those shifts and the actual shifts in the ^{15}N-HMQC-NOESY spectrum. To take into account this possible shift difference, you need to edit the spectrum-specific shifts of the patterns. This can be done either manually (where for each pattern the chemical shifts of frequencies are adjusted based on displayed intrapattern peaks) or automatically.

Select the **Assign/Spin System/Auto Update Specific Shifts** menu item. Select the **noe** spectrum and click **All**. Set the tolerances to **0.02**, **0.04** and **0.1**. Click **OK**.

*In few minutes the spectrum-specific chemical shifts are set for all the patterns. You can see the results by using the **Assign/Report Spin System** menu item for e.g. the first pattern:*

```
LISTING FOR PATTERN pa1

 comment            :  fromproto1
 color              :  Red
 root frequency     :  8.451

             Frequencies
             -----------
 generic       specific     assignment
  shift                shifts
              1     2     3     4     5     6     7     8     9
            hsqc tocsy noe null null null null null null
 8.451      8.451 8.451 8.446 8.451 8.451 8.451 8.451 8.451 8.451     1:*_*:HN
 123.444      123.444 123.444 123.447 123.444 123.444 123.444 123.444 123.444 123.444     1:*_*:N
 3.739      3.739 3.739 3.734 3.739 3.739 3.739 3.739 3.739 3.739     1:*_*:HX
 2.037      2.037 2.037 2.023 2.037 2.037 2.037 2.037 2.037 2.037     1:*_*:HX
 0.816      0.816 0.816 0.803 0.816 0.816 0.816 0.816 0.816 0.816     1:*_*:HX

       The neighbor probabilities
       --------------------------

    Pattern              Probability

     The residue type probabilities
     ------------------------------

    Residue              Probability
      ILE                 1.000
      VAL                 1.000
      THR                 0.833
      ARG                 1.000
      LYS                 1.000
      LEU                 1.000

   (i-1) Frequencies from protopattern  1
   ----------------------------------------
```

You must update the root frequency attribute of the patterns:

Select the **Assign/Spin System/Copy Specific Shift to Generic** menu item. Set **Patterns** to **All** and **Spectrum** to **NOE**. Click **OK**.

Now select the **Assign/Spin System/Auto Root** menu item, set **Patterns** to **All**, and click **OK**.

18. Performing neighbor searches

Select the **Assign/Neighbor/Find Neighbor Via 3D N-15 NOE** menu item. Set these values:

Experiment	**noe**
Candidate (NOE) Dimension	**D2**
Frequency Collapse Tolerance	**0.04**
Specify Patterns	**All Patterns**
Normalize Scores	**on**
Store Scores	**Overwrite Old**
Number of Neighbors to Save	**12**
Output Level	**Low**

Click **More...** and set these values:

Number of Frequencies to Use in Pattern	**6**
First Type	**H**
High	**12.0**
Low	**6.0**
Second Type	**N**
High	**130.0**
Low	**90.0**
Candidate Frequency Range	
High	**12.0**
Low	**-2.0**
Minimum # of NOE Peaks	**2**

Click **OK**.

*In few seconds the neighbor search is done. There are several ways to check for the result of the run; using the previously described **Assign/Report Spin System** menu item now will result in output such as:*

```
LISTING FOR PATTERN pa1

 comment            :  fromproto1
 color              :  Red
 root frequency     :  8.446

          Frequencies
          -----------
 generic                specific                      assignment
  shift                  shifts
              1     2     3     4     5     6     7     8     9
            hsqc tocsy noe null null null null null null
 8.446       8.451 8.451 8.446 8.451 8.451 8.451 8.451 8.451 8.451     1:*_*:HN
 123.447       123.444 123.444 123.447 123.444 123.444 123.444 123.444 123.444 123.444     1:*_*:N
 3.734       3.739 3.739 3.734 3.739 3.739 3.739 3.739 3.739 3.739     1:*_*:HX
 2.023       2.037 2.037 2.023 2.037 2.037 2.037 2.037 2.037 2.037     1:*_*:HX
 0.803       0.816 0.816 0.803 0.816 0.816 0.816 0.816 0.816 0.816     1:*_*:HX

       The neighbor probabilities
       --------------------------

    Pattern              Probability
      pa12                 0.2500
      pa28                 0.1875
      pa51                 0.1875
      pa48                 0.1250
      pa50                 0.1250
      pa62                 0.1250

     The residue type probabilities
     ------------------------------

    Residue              Probability
      ILE                  1.000
      VAL                  1.000
      THR                  0.833
      ARG                  1.000
      LYS                  1.000
      LEU                  1.000

   (i-1) Frequencies from protopattern  1
   ----------------------------------------
```

*You can also use the **Assign/Neighbor/List Neighbors** menu item:*

Select the **Assign/Neighbor/List Neighbors** menu item. Select **pa1** from the **List of Patterns** and leave **Order** set to **i - i+1**. Click **OK**.

The output will be similar to:

```
   The possible neighbors (i - i+1) for pattern pa1
are:
     pattern pa12    with probability: 0.2500
     pattern pa28    with probability: 0.1875
     pattern pa51    with probability: 0.1875
     pattern pa48    with probability: 0.1250
     pattern pa50    with probability: 0.1250
     pattern pa62    with probability: 0.1250
```

Or you can visually inspect the neighboring patterns:

Go to the **Spinsystems** table and select the first pattern by clicking it. Then select the **Spinsystem/Show i+1 Neighbors Via Strip Plot** menu item.

Seven strips appear on the screen, containing plots of the region containing the frequencies of pattern pa1, and the neighboring patterns: pa12, pa28, pa48, pa50, pa51, and pa62. Also, the output window shows:

```
Strip plot of pattern 1 with neighbors:
     pa12    0.2500
     pa28    0.1875
     pa51    0.1875
     pa48    0.1250
     pa50    0.1250
     pa62    0.1250
```

The next step is to generate possible sequence-specific assignments for the patterns, that is, to compare spin-system type and neighbor-probability information with the primary sequence and make suggestions about which pattern belongs to which particular amino acid in the sequence. This can be done in FELIX through the ***Assign/Sequential*** *menu items. Here we show one approach, using the* ***Assign/ Sequential/Systematic Search*** *menu item; other approaches can be found in Chapter 2, Tasks, in the FELIX User Guide.*

Since the ^{15}*N-HMQC-TOCSY spectrum does not contain spin systems from prolines therefore we need to find stretches of sequential assignments between the Pro residues. The first such one in the sequence is between residues 4 and 8. Certainly, if one has the Pro spin systems detected, then this limitation does not exist.*

19. Generating sequence-specific assignments for the stretch of residues 4-8

Select the **Assign/Sequential/Systematic Search** menu item and set these values:

Min individual assignment prob	**0.8**
Min Neighbor Prob Score	**0.15**
First residue (#) to consider	**4**
Last residue (#) to consider	**8**
Min Length of Assigned Stretches	**4**
Output level	**Low**

Leave the other parameters at the default values and click **OK**.

After few seconds you will see a listing of several possible assignments, the beginning of which would look like:

```
Sorting out and storing the generated assignments
   Assignments left :(          8  )

assignment #     1 -- length =   5 residues
   ... stretch of residues =   4 -   8 total scores :     4.95    1.83
   Residues : ASP-_4   ALA_5    ILE_6    ASN_7    ALA_8
   Patterns :       36      37       4       44       58
   Scores   :     0.99    0.99    0.99    0.99    0.99
   I>I+1    :     0.33    0.50    0.33    0.67

   assignment #     2 -- length =   4 residues
   ... stretch of residues =   5 -   8 total scores :     3.96    2.00
   Residues : ALA_5    ILE_6    ASN_7    ALA_8
   Patterns :       33       4       44       58
   Scores   :     0.99    0.99    0.99    0.99
   I>I+1    :     1.00    0.33    0.67

...
The Pattern Suggest Assignment took 2 seconds!
```

Also, a new spreadsheet is activated: ***Stretches*** *where stretches of possible sequential assignment are stored.*

20. Showing the first possible sequential assignment

Now you can inspect the first possible stretch.

Go to the **Stretches** table and select the first row by clicking it, then click the **ND Strip Plot** icon.

In few seconds a strip plot appears which contains five vertical strips—along the H_N-N frequencies of the patterns pa36, pa37, pa4, pa44, and pa58. Also, a message is printed in the output window:

```
  . Strip plot of stretch # 1
The patterns in the stretch are:
                            pa36
                            pa38
                            pa4
                            pa44
                            pa58
```

21. Generating sequence-specific assignments for the stretch of residues 4-8 via simulated annealing

Next you try the other method available for sequential assignment: simulated annealing.

Select the **Assign/Sequential/Simulated Annealing** menu item. Set these values:

Min Individual Assignment prob	**0.8**
First Residue (#) to Consider	**4**
Last Residue (#) to Consider	**8**
Discard Previous Assignments	**False**
Store Generated Assignments	**False**
Output Level	**High**

Leave the other parameters at the default value (**1.0**) and click **OK**.

The output in the output window should be similar to this:

```
SA completed: initial energy 6.500 and final energy 2.000(niter 30)
 new sequence (energy 1.875):
  10 33 4 44 58

 Suggested assignment:
ASP4 -> pa10   Type score 1.000        Neighbor probability 0.125
```

```
ALA5 -> pa33   Type score 1.000        Neighbor probability 1.000
ILE6 -> pa4    Type score 1.000        Neighbor probability 0.333
ASN7 -> pa44   Type score 1.000        Neighbor probability 0.667
ALA8 -> pa58   Type score 1.000
The Pattern Suggest Assignment took 3 seconds!
```

Now you can inspect this assignment too.

Go to the **Spinsystems** table and first select the tenth row (**pa10**). Then <Ctrl>-click to select the rows containing **pa33**, **pa4**, **pa44**, and **pa58**.

Click the **ND Strip Plot** icon.

In the output window a message appears:

```
  Strip plot of pattern 10
Strip plot of pattern 33
Strip plot of pattern 4
Strip plot of pattern 44
Strip plot of pattern 58
```

and five vertical strips are displayed in the frame.This solution was not found in systematic search, since the neighbor probability measure between pa10 and pa33 is 0.125, which is lower than the cutoff we set (0.15).

Using the simulated annealing and systematic search methods for sequential assignment one can then assign the patterns to specific residues.

22. Exiting FELIX

To exit FELIX, select the **File/Exit** menu item.

Lesson 3: Heteronuclear triple resonance 3D assignment strategy

This lesson takes you through typical steps in the assignment of a doubly-labelled protein. The data set consists of the HNCACB and CBCA(CO)NH spectra of the ^{13}C- and ^{15}N-enriched RNA-binding domain of hnRNP C from Luciano Mueller (Bristol-Myers Squibb, Princeton: see Wittekind 1992).

The topics covered in this lesson are:

- Database setup.
- Spin-system detection using triple resonance spectra.
- Spin-system verification.
- Spin-system identification.
- Sequence-specific assignment.

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in “Setting up tutorial files” in the preface, *How To Use This Book*.

The files for this lesson are all located in the **Assignment\Lesson3** folder

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, Navigate to your working directory. If you used the default suggestion, this will be: **C:\Felix_Practice\Assignment\Lesson3**.

Enter **hnrp** and select **OK** to build a new database file.

3. Setting up the database

Select the **Assign/Project** menu item. When a dialog box appears informing you that no project was found in the database and asking if you want to build one, click **OK**.

In the next control panel, enter a name (e.g., **rnp**:**project**).

In the next control panel you need to select a molecule, in this case an extended chain of hnRNP C:

Selection	**hnrnp.car**
File Type	**Insight Molecule (*.car)**

Click **OK** to build the entities and read in the molecule.

This procedure typically takes several seconds; a meter on the status bar shows the progress.

4. Defining a library

After the setup is successfully completed, you should define a library. The library is an ASCII file, as described in the Assign/Define Library

section of Chapter 5, Assign User Interface, in the FELIX User Guide. FELIX contains a standard library (pd.rdb) which you should read in.

In the next control panel (Library) Select **Define Library from File.** In the next control panel, select **pd.rdb**, which stands for protein-DNA library. A few seconds later the setup procedure is finished.

5. Adding experiments to the projects

Select the **Assign/Experiment** menu item to define new experiments in the assignment database. When the control panel appears with names of matrices, select **hncacb.mat** (the **HNCACB** spectrum).

Click **OK**.

Set these parameter values for the plot using the 3D DISPLAY PARAMETERS control panel:

Contour Threshold	**0.3**
Color Scheme	**Blue/Green**
Axis type D1	**ppm**
Axis type D2	**ppm**
Axis type D3	**ppm**
D1 Scale	**1**
D2 Scale	**1**
D3 Scale	**1**

Leave the other parameters at their default values and select **Apply**.

Click **OK** when the message box appears.

*If you want, you can change the display parameters using the **Experiment/Change Attribute** menu item in the **Experiment** table.*

*The program plots a density plot or contour plot of the HNCACB using the parameters you defined. Note, since by default this is the first plane, that possibly no cross peaks may be seen, but you can select a different plane (or different view) using the **New Plane** button later.*

Now another control panel appears.

Set the parameters to these values:

Title	**hnccb**
Use Default Names	**on**
Type	**3D CBCANH**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Carbon**
D2 Nucleus	**Nitrogen**
D3 Nucleus	**Proton**
D1 Tol	**0.15**
D2 Tol	**0.15**
D3 Tol	**0.02**
D1 Fold	**No**
D2 Fold	**No**
D3 Fold	**No**
W1	**D3**
W2	**D2**
W3	**D1**
W1-W2 Transfer	**J-coupled**
# of J Steps	**1**
W2-W3 Transfer	**J-coupled**
# of J Steps	**1**

Click **OK**.

It is important to define the spectrum-specific tolerances, since they are used in many automated and semi-automated procedures.

A new window containing an Experiments table is open and displayed to the left of the spectral window. Note that, by default, whenever a new window (table or spectral) is open, FELIX automatically re-arranges the layout of the windows. You can turn off this feature by selecting Preference/Frame Layout from the main menu and set Action to None. You can also do the automatic re-arrangement at anytime by selecting Window/Auto Arrange.

Note: When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

6. Adding the CBCA(CO)NHN spectrum to the project

Activate the **Experiments** table. Select the **Experiments/ Add** menu item.

Select **cbcaconh.mat** and set these values:

Contour Threshold	**0.3**
Color Scheme	**Magenta**
Number of Levels	**16**
Axis Type	**ppm**
Negative Levels	**Off.**

Click **OK**.

In the next control panel, set the parameters to these values:

Title	**cacbconh**
Use Default Names	**on**
Type	**3D CBCA(CO)NH**
Temperature	**298**
pH	**7**
Solvent	**Water**
D1 Nucleus	**Carbon**
D2 Nucleus	**Nitrogen**
D3 Nucleus	**Proton**
D1 Tol	**0.15**
D2 Tol	**0.15**
D3 Tol	**0.02**
D1 Fold	**No**
D2 Fold	**No**
D3 Fold	**No**
W1	**D3**
W2	**D2**
W3	**D1**
W1-W2 Transfer	**J-coupled# of J**
Steps	**1**
W2-W3 Transfer	**J-coupled # of J**
Steps	**1**

Click **OK**.

7. Reading in the peaks

In the **Experiments** table select the **hncacb** experiment (click the first row) and click the **Draw** icon. This draws the hncacb spectrum in the spectral frame.

Activate the spectral frame and select the **File/Import/ Peaks** menu item. Select **hncacb.xpk** and leave the **FELIX Peak Table Name** parameter at its current status (**xpk:hncacb**). Click **OK**.

When the query box asks you about overwriting the entity, click **OK**.

This action reads in the peaks and also displays them as a peak table.

Now select the **cbcaconh** experiment in the **Experiments** table and click the **Draw** icon to plot it.

Activate the spectral window and select the **File/Import/ Peaks** menu item. Select **cbcaconh.xpk** and click **OK**. In the new query box asking about overwriting the entity, click **OK**.

Now you have a full peak set defined for both experiments. Close the two peak tables by selecting File/Close from their menus.

8. Selecting the HNCACB spectrum

Now reselect the **hncacb** experiment from the **Experiments** table and click the **Draw** icon to display the spectral window.

The next step is the collection of prototype patterns, i.e., sets of frequencies, which later are promoted to patterns and assigned to specific amino acid residues. The commands related to prototype patterns are in the ***Assign*** *pulldown in the third subsection. Since we have the HNCACB and the CBCA(CO)NH spectra in our project, we demonstrate the use of one of the triple resonance prototype pattern collection methods.*

9. Performing a prototype pattern detection

Activate the spectral window and select the **Assign/Collect Prototype Patterns** menu item. In the control panel, select the **Triple Resonance** option and click **OK**.

In the following control panel, select the **CBCANH + CBCA(CO)NH** option and click **OK**.

Set these parameter values in the third control panel:

Use Default Experimentson
CA peak intensity sign +
CB peak intensity sign -

CA peak intensity sign +
CB peak intensity sign +

Tolerances	
HN	**0.02**
C	**0.2**
N	**0.2**
Number of iterations	**5**
Tolerance factor	**1.2**
Output level	**Low**

Click **OK**.

After a couple of minutes, the prototype pattern collection is finished, and the following information appears in the output window:

```
The 3D protopattern detection took 154 seconds for 5 iteration
and the total number of prototype patterns now : 79
The number of unclassified peaks in cbcaconh.mat is: 134
The number of unclassified peaks in hncacb.mat is: 231
```

A new table of prototype patterns also appears. Comparing these prototype patterns with the spin systems assigned previously (1) we confirm that 82% of the spin systems were picked correctly.

10. Visually inspecting several prototype patterns

From the **Protopatterns** table select the first protopattern and then click the **Zoom** icon.

The region containing the four HNCACB peaks of the first protopattern is displayed.

In order to see the HNCACB and CBCACONH peaks simultaneously when you browse the prototypes, you use two connected spectral windows as follows.

11. Connecting the HNCACB and CBCACONH spectra

For the following action you need to have room for two spectral frames.

Activate the spectral window and select the **Window/New Frame Layout** menu item. In the control panel, set the **New Layout** parameter to **2 Frames Up/Down**. Click **OK**.

Two spectral frames are displayed and the other table windows are reorganized automatically. Since the up/down layout of two spectral windows is not the default layout of FELIX, you have to toggle off the automatic rearrangement option in order not to mess up your layout when a new window

Select the **Preference/Frame Layout** menu item. Set **Action** as **None** for **Auto. Frame Organization**. Select **OK**.

Now activate Frame 2 and then select the **cbcaconh** experiment in the Experiments table. Click the **Draw** icon.

Next activate Frame 1 and click the **Plot** icon.

While Frame 1 is activated, select the **Preference/Frame Connection** menu item. In the control panel, leave **1** for the **First Frame** and **2** for the **Second Frame** parameters. Select **D1-D2-D3 <=> D1-D2-D3** and click **OK**.

Now the two spectra are connected in all three dimensions. Now you can navigate in the first frame and the display is updated in the second frame, too. For example, if you zoom on a prototype pattern in the first frame, the same region is displayed in the second frame, too.

You can use different activities to browse through the spectrum in Frame 1, and the same action also takes place for Frame 2.

12. Correcting the prototype patterns

In this section you find out how to make corrections to the automatically detected prototype patterns. To do this, you need to zoom on a prototype pattern and then inspect the frequencies (which are drawn through the peaks).

Go to the **Protopatterns** table, select the first prototype pattern, and click the **Zoom** icon.

Alternatively, you can double-click the first row, since the default action for double-clicking is to zoom. You can change the default action using the popup on the combo box of the table.

You can see that the first prototype pattern is correct. Now zoom on the second prototype pattern, and you can see that this one is also correct. When you zoom on the third prototype pattern, you can see that only three carbon frequencies were found and that there is a well-defined peak at 64.1, 121.8, 9.4 ppm. If you look at the prototype pattern in the table you can see that the CA(i) was missed, and most likely this peak contains that frequency (64.1 ppm). Here, you will add that frequency:

Go to the **Protopatterns** table and select the **ProtoPattern/ Add Frequency via Cursor** menu item.

With the crosshair cursor, click the CA(i) peak that was missed. In the ADD FREQUENCY TO PROTOPATTERN 3 control panel, set the **Frequency** to **F1 64.06** and the **Nucleus 1** to **CA**. Click **OK**.

Now the prototype pattern is redrawn on the screen with four carbon frequencies and the table is updated: there is now a CA frequency for the third protopattern.

You would typically go through the full set of prototype patterns and make similar adjustments. Also, you can edit the frequencies in the table directly or by using the ***Assign/Edit Prototype*** *menu item. You can also delete spurious protopatterns through the table or through the* ***Assign/Edit Prototype*** *menu item.*

After the prototype patterns are cleaned up you can promote them to patterns.

13. Promoting the prototype patterns to patterns

Select the **Assign/Promote Prototype Patterns** menu item. In the first control panel, select the **Copy Prototype Patterns to Spin Systems (Patterns)** option and click **OK**.

In the next control panel, set the **Sequential Tolerances for C** to **0.25,** set the **Find Prolines** to **Yes**, and leave the **Compare Similarities** parameter as **No**. Click **OK**.

You can follow the progress in the output window: in first stage you can see that the regular spin systems are to be promoted—i.e., non-prolines. Then FELIX tries to find spin systems that can be interpreted as prolines—i.e., such protopatterns that contain a $C_{\alpha,i-1}$ *in the 60–66 ppm range and a* $C_{\beta,i-1}$ *in the 28–34 ppm range and no appropriate* H_N *and N. In third stage FELIX tries to find the possible sequential connectivities and stores them with the newly generated patterns as probabilities. After a few minutes 86 new patterns are generated and the table*

*containing them is displayed (**Spinsystems**). You can either inspect the patterns in the table or use the **Assign/Report Spin System** menu item:*

```
LISTING FOR PATTERN pa1
 comment            :  fromproto1
 color              :  Red
 root frequency     :  9.637

              Frequencies
              -----------
 generic       specific           assignment
  shift                shifts
              1     2     3     4     5     6     7     8     9
            hncacb cbcaconh null null null null null null null
 9.637      9.637 9.637 9.637 9.637 9.637 9.637 9.637 9.637 9.637     1:*_*:HN
 129.947      129.947 129.947 129.947 129.947 129.947 129.947 129.947 129.947 129.947     1:*_*:N
 61.505      61.505 61.505 61.505 61.505 61.505 61.505 61.505 61.505 61.505     1:*_*:CA
 34.436      34.436 34.436 34.436 34.436 34.436 34.436 34.436 34.436 34.436     1:*_*:CB

       The neighbor probabilities
       --------------------------

    Pattern              Probability
      pa4                 0.3333
      pa37                0.2222
      pa38                0.2222
      pa70                0.2222

     The residue type probabilities
     ------------------------------

    Residue              Probability

   (i-1) Frequencies from protopattern  1
   ----------------------------------------

    CA(i-1)    54.484 ppm
    CB(i-1)    33.110 ppm
```

14. Scoring the patterns

Select the **Assign/Residue Type/Score Residue Type** menu item. Check **Use All Pattern**. Set the **Scoring Method** to **CACB only.** Select **Store** for **Database**. Click **OK**.

*After few seconds all 86 patterns are scored and the residue type probabilities are stored. Using the **Assign/Report Spin System** menu item for the first pattern gives a similar result:*

```
LISTING FOR PATTERN pa1

 comment            :  fromproto1
 color              :  Red
 root frequency     :  9.637

           Frequencies
           -----------
 generic    specific        assignment
  shift                  shifts
              1     2     3     4     5     6     7     8     9
             hncacb cbcaconh null null null null null null null
 9.637       9.637 9.637 9.637 9.637 9.637 9.637 9.637 9.637 9.637     1:*_*:HN
 129.947       129.947 129.947 129.947 129.947 129.947 129.947 129.947 129.947 129.947     1:*_*:N
 61.505       61.505 61.505 61.505 61.505 61.505 61.505 61.505 61.505 61.505     1:*_*:CA
 34.436       34.436 34.436 34.436 34.436 34.436 34.436 34.436 34.436 34.436     1:*_*:CB

       The neighbor probabilities
       --------------------------

    Pattern              Probability
      pa4                 0.3333
      pa37                0.2222
      pa38                0.2222
      pa70                0.2222

     The residue type probabilities
     ------------------------------

    Residue              Probability
      MET                 0.001
      VAL                 0.479
      LYS                 0.009
      PRO                 0.471
      PHE                 0.001
      ILE                 0.037
      TYR                 0.002

   (i-1) Frequencies from protopattern  1
   ------------------------------------------

    CA(i-1)    54.484 ppm
    CB(i-1)    33.110 ppm
```

15. Generating sequence-specific assignments for patterns

Select the **Assign/Sequential/Systematic Search** menu item. Set the **Min Individual Assignment Prob** to **0.1** and set the **Last residue (#) to Consider** to **94**. Set the **Min Length of Assigned Stretches** to **3**. Select **Low** for **Output level** and leave the other parameters at the default value. Click **OK**.

After few seconds you will see many possible assignments; the beginning of the output will look like:

```
assignment #     1 -- length =  10 residues
   ... stretch of residues =  69 -  78 total scores :     5.38    3.74
Residues : GLY_69   GLU-_70  ASP-_71  GLY_72   ARG+_73  MET_74   ILE_75   ALA_76
  Patterns :       49       64       58       41       77       20       22        2
  Scores   :     0.99     0.17     0.26     0.99     0.23     0.19     0.43     0.99
  I>I+1    :     1.00     0.27     0.43     0.33     0.33     0.33     0.38     0.33
  Residues :    GLY_77   GLN_78
  Patterns :       31       76
  Scores   :     0.99     0.14
  I>I+1    :     0.33

assignment #     2 -- length =   9 residues
   ... stretch of residues =  70 -  78 total scores :     4.37    2.80
Residues : GLU-_70  ASP-_71  GLY_72   ARG+_73  MET_74   ILE_75   ALA_76   GLY_77
  Patterns :       55       58       41       77       20       22        2       31
  Scores   :     0.13     0.26     0.99     0.23     0.19     0.43     0.99     0.99
  I>I+1    :     0.33     0.43     0.33     0.33     0.33     0.38     0.33     0.33
  Residues :    GLN_78
  Patterns :       64
  Scores   :     0.16

assignment #     3 -- length =   9 residues
   ... stretch of residues =  70 -  78 total scores :     4.35    2.80
Residues : GLU-_70  ASP-_71  GLY_72   ARG+_73  MET_74   ILE_75   ALA_76   GLY_77
  Patterns :       55       58       41       77       20       22        2       31
  Scores   :     0.13     0.26     0.99     0.23     0.19     0.43     0.99     0.99
  I>I+1    :     0.33     0.43     0.33     0.33     0.33     0.38     0.33     0.33
  Residues :    GLN_78
  Patterns :       76
  Scores   :     0.14
```

*The scoring stores the results in the database as stretches. At the end of the action the stretches are shown in tabular form. This table can be closed and reopened using the **Edit/Stretch** menu item. Next, we show how to use stretches in helping with the assignment procedure.*

16. Reviewing the first stretch

First increase the size of the frame containing the **hncacb** experiment (Frame 1). Now go to the **Stretches** table and select the first row by clicking it, then click the **ND Strip Plot** icon.

You will see the strip plot of patterns 49, 64, 58, 41, 77, 20, 22, 31, and 7. I

17. Setting the sequence-specific assignments for patterns 49, 64, 58, 41, 77, 20, 22, 2, 31, and 76

Go to the **Stretches** table. With the first row highlighted, select the **Stretch/Assign One Stretch** menu item. Start the stretch at **GLY_69** and leave the **Assign Frequencies** on. Click **OK**.

During execution the following lines appear in the output window:

```
  Pattern pa49 assigned to residue 1:GLY_69.
Pattern pa64 assigned to residue 1:GLU-_70.
Pattern pa58 assigned to residue 1:ASP-_71.
Pattern pa41 assigned to residue 1:GLY_72.
Pattern pa77 assigned to residue 1:ARG+_73.
Pattern pa20 assigned to residue 1:MET_74.
Pattern pa22 assigned to residue 1:ILE_75.
Pattern pa2 assigned to residue 1:ALA_76.
Pattern pa31 assigned to residue 1:GLY_77.
Pattern pa76 assigned to residue 1:GLN_78.
```

When you are done you can check the resulting pattern using the ***Assign/Report Spin System*** *menu item. For example, pattern 58 would yield a similar result:*

```
LISTING FOR PATTERN pa58

 comment            :  fromproto58
 color              :  Red
 root frequency     :  7.883

             Frequencies
             -----------
 generic       specific        assignment
  shift                shifts
              1     2     3     4     5     6     7     8     9
            hncacb cbcaconh null null null null null null null
 7.883      7.883 7.883 7.883 7.883 7.883 7.883 7.883 7.883 7.883     1:ASP-_71:HN
 122.905      122.905 122.905 122.905 122.905 122.905 122.905 122.905 122.905 122.905     1:ASP-_
71:N
 57.338     57.338 57.338 57.338 57.338 57.338 57.338 57.338 57.338 57.338     1:ASP-_71:CA
 41.030     41.030 41.030 41.030 41.030 41.030 41.030 41.030 41.030 41.030     1:ASP-_71:CB

       The neighbor probabilities
       --------------------------

    Pattern              Probability
      pa3                 0.1429
      pa18                0.2857
      pa41                0.4286
```

```
   pa72                 0.1429

  The residue type probabilities
  ------------------------------

 Residue              Probability
   ASP                 0.257
   PHE                 0.244
   ILE                 0.050
   LEU                 0.214
   TYR                 0.235

(i-1) Frequencies from protopattern  58
-----------------------------------------

 CA(i-1)   54.775 ppm
 CB(i-1)   30.755 ppm
```

18. Exiting FELIX

To exit FELIX, select the **File/Exit** menu item.

5 Using Autoscreen

Lesson 1: Analyzing 2D ^{15}N–HSQC spectra of calcyclin

This lesson presents the basic steps of SAR by NMR analysis using the Autoscreen module of FELIX. In this lesson you use a set of three ^{15}N-enriched HSQC ser files of calcyclin acquired on a Bruker spectrometer.

The topics covered in this lesson are:

- Setting up and adding experiments to a project.
- Processing a control experiment and setting up a spectral display.
- Processing and scoring test experiments.
- Analyzing and editing scoring results.
- Exporting and displaying results.

1. Setting up for the lesson

> If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*. The files for are located in the **Autoscreen\Lesson1** folder.

The files are briefly described below:

Table 1 Files in the sar directory

File	Purpose
\1*	ser and parameter files of control experiment.
\2*	ser and parameter files of first test experiment.
\3*	ser and parameter files of second test experiment.
analysis\exps.txt	A list of experiments, used to add experiments to the Autoscreen project.
analysis\bmrb_assign.tbl	Resonance assignment of some of the HSQC peaks.
str\demo.pdb	PDB file of the demo molecule.

The exps.txt file is a list of input experiments and their associated structural files, with each line specifying the experiment ID, ser file (with path relative to /usr/people/cpeng/sarnmr/test/scripps/; see Step 3 for project paths), file type, structural filename (optional), and comments (optional):

```
calcyclin     1/ser  serdemo       control
annexinXI_34  2/ser  sertest.car   test-1
annexinXI_48  3/ser  sermols.txt
```

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, navigate to your working directory. If you used the default suggestion, this will be: **C:\Felix_Practice\Autoscreen\Lesson1**.

Enter **test** and click **OK** to build a new database file.

Tip: If you set up an Autoscreen project in the previous session and want to use the same spectrum display and scoring parameters as in that project, click **OK** in the RESTORE LAST SESSION dialog box.

Remember that spectrum-processing parameters are *not* inherited from session to session.

3. Setting up the project

Select the **Autoscreen/Project** menu item from the FELIX menu bar. When you see the AUTOSCREEN PROJECT control panel, leave the default parameters unchanged (**sar** and **2D**) and click **OK**.

You should see this in the status bar:

```
Created new Autoscreen project 'sar'.
```

Note: Currently a project name is limited to less than nine lower-case alphanumeric characters. You can create only one Autoscreen project in a database file. Once a project is finished, you can select the **File/New** menu item to open a new database after saving the current one, and then repeat this step to create a new project.

The VERIFY DIRECTORIES control panel, which appears next, allows you to verify some important paths used to access or save the following files:

- *Raw spectrometer data files.*
- *Processed matrix files.*
- *ASCII files, including file list and results.*

For this example, you are using relative paths for all experiments and molecular files, so it is important to verify the project paths.

Make sure that the paths in the VERIFY DIRECTORIES control panel are similar to the following:

Spectrometer Data:
C:\Felix_Practice\Autoscreen\Lesson1

Matrix Files:
C:\Felix_Practice\Autoscreen\Lesson1\analysis

ASCII Text Files:
C:\Felix_Practice\Autoscreen\Lesson1\analysis

*You can click **Browse** next to any of the paths to select a directory interactively or you can directly enter a directory name.*

Click **OK**.

A new window containing an empty Experiments table is open and displayed to the left of the spectral window.

Note: By default, whenever a new window (table or spectral) is open, FELIX automatically re-arranges the layout of the windows.

You can turn off this feature by selecting **Preference/Frame Layout** from the main menu and set **Action** to **None**. You can also do the automatic re-arrangement at anytime by selecting **Window/Auto Arrange**.

Remember: When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

Table 2 Description of items in the Autoscreen Experiments Table

Column	Description
id	ID of the experiment.
score	Total score of the experiment.
thresh	Threshold used for peak picking if scored.
status	Status of the experiment, with 0 standing for nonprocessed, 1 for processed, 2 for scored, and 9 for control spectrum.
fid	File name of the FID file, if any.
type	File type of the FID file, with ser for Bruker serial file, fid for Varian FID file, mat for FELIX matrix, 2rr for Bruker processed file, var for Varian processed file, spc for NMR Compass file, ft2 for NMRPipe file, and nmr for TRIAD file.
struc	Filename of a molecule, with extension .pdb standing for PDB file, .car for CAR file, and .mol for MDL file. It can also be filename of a list of molecules if another extension is used.
comment	Comments. (You can enter the concentration here for titration spectra.)

> **Tip:** If you want to change the project paths again, use the **Edit/ Verify Directories** menu item from the table or the **Autoscreen/ Experiment/Verify Directories** item on the main menu bar.

4. Adding experiments to a project

The following steps demonstrate three ways of adding experiments to an Autoscreen project.

Option 1—Add one experiment at a time

Activate the spectral window and select the **Autoscreen/ Experiment/Add One** menu item.

In the ADD ONE EXPERIMENT control panel, select **Bruker (ser)** as the **Spectrum File Type**, enter **Control** as the **Experiment ID**, and fill in the **Comment** with **This is the control experiment**.

If you want, you may turn **on** the **Molecule** toggle and click the **Browse** button. When the SELECT MOLECULAR FILE OR FILE LIST file browser appears, select **demo.pdb** and click **OK** to return to the ADD ONE EXPERIMENT control panel.

Finally, select the **ser** file under the directory **1**, and click **OK** to add this experiment to the project.

The Autoscreen Experiments Table is updated with the newly added experiment.

The ADD ONE EXPERIMENT control panel is displayed again for you to add another experiment. Click **Cancel** to close it.

Caution: If you select a molecule file interactively, be sure to do so before selecting the **ser** file. Otherwise, FELIX does not "remember" that you selected the **ser** file and you will have to do it again.

Option 2—Add experiments from all files in a directory

First clean up from your test of the previous option:

Highlight the row of interest (or all rows if you added more than one experiment) in the Autoscreen Experiments Table and select the **Edit/Delete Experiments** menu item to remove the experiment.

Next, add experiments from all files.

Activate the spectral window and select **Autoscreen/ Experiment/Add All Files**. In the ADD ALL EXPERIMENTS control panel, change **To Dir** to **3**, the highest experiment number. Then click **OK**.

All three experiments are added to the project, with the first experiment taken as the control spectrum.

Tip: This function expects numbered Bruker experiments. If the experiments are not consecutively numbered, you can instruct the program to skip one or more between every two experiments, or it will automatically ignore nonexistent experiments.

Option 3—Add all experiments listed in a file

First clean up from your test of the previous option:

Highlight all rows in the Autoscreen Experiments Table. From the table, select the **Edit/Delete Experiments** menu item to remove all the experiments.

Next, add experiments by reading a list in a file.

Activate the spectral window and select the **Autoscreen/ Experiment/Add From File List** menu item and, in the ADD EXPERIMENTS FROM FILE LIST control panel, select the file **exps.txt** and click **OK**.

This adds three experiments to the project, using the experiment list in the exps.txt file. These experiments are the ones that we will use in subsequent processing and scoring in this lesson.

5. Processing a control spectrum

Unless you are using processed data, for example, FELIX matrices or Bruker 2rr files, one of the most important steps in using Autoscreen is processing the control spectrum. The processing parameters used during this procedure are used for the subsequent processing of all other test experiments.

Highlight the **calcyclin** experiment in the Autoscreen Experiments Table and select **Action/Process Control Spectrum** from the menu bar of the table.

Next you are guided through the processing of this 2D experiment. The procedure is similar to standard 2D processing in FELIX.

Keep the default parameters unchanged and click **OK** in the 2D HEADER INFORMATION and 2D ACQUISITION INFORMATION control panels.

In the 2D DATA PROCESSING control panel, which appears next, select **Automatic** for **Phasing Mode** and **Facelift** for **Baseline Correction**. click **OK**.

*You will use the automatic phasing function (called **PAMPAS**) and baseline-correction function (called **FACELIFT**) after the Fourier transform.*

Tip: PAMPAS automatically determines phasing parameters for a processed matrix. You will be prompted to set parameters for it later. If you want to phase interactively, select **Interactive** as the **Phase Mode** and enter **3** for **Fid to Phase**.

Click **OK** in the SINEBELL PARAMETERS control panel. If it warns you about overwriting an existing file, click **Overwrite**.

In the 2D DATA PROCESSING control panel, check **Linear Prediction**, select **Automatic** as the **Phasing Mode**, and **Facelift** as the **Baseline Correction**. Click **OK**.

In the GENERAL LINEAR PREDICATION control panel, change the **Number of Coefficients** to **8** and click **OK**.

Click **OK** in the SINEBELL PARAMETERS control panel.

In the AUTOPHASING (PAMPAS) PARAMETERS control panel, make sure **Correct D1** and **Correct D2** are checked. Under **Excluded Areas**, check **#1**. Click the **Cursor** button on the same line to set the excluded area interactively.

The purpose of this action is to exclude the water signals while determining the phase parameters. The cursor changes to a cross, allowing you to define a range to exclude for D1.

Click at a point between the real peaks and the water signals, keep the left mouse button depressed, and drag the cursor to the right limit of the spectrum (the Y coordinates do not matter), then release the mouse button.

The same control panel appears again with the excluded range displayed in points (for example from 374 to 512). If necessary, you can edit these numbers in the entry boxes.

Click **OK**.

The spectrum is automatically phased in both dimensions. In the output window, the determined phase parameters and other information are displayed.

Note:

- If you choose to apply PAMPAS to the control spectrum, it is applied to all test spectra later. Since it determines phase parameters for each individual spectrum (instead of applying the same phase parameters as for the control spectrum), spectra with different phase errors are not a problem. For more reliable phasing results, it is important to exclude noise when determining phase parameters.
- If you choose to extract a portion of the spectrum during processing (e.g., to extract the left half) and the truncation happens on the water ridge, autophasing restores the truncated water spectrum on the left side of the spectrum. To avoid this, use interactive phasing instead of autophasing.

Finally, the BASELINE CORRECTION (FACELIFT) PARAMETERS control panel appears. Make sure **D1** and **D2** are checked for **Correction Dimension** and click **OK**.

The processed HSQC spectrum is now displayed as contours.

6. Setting display reference and display limits

The spectral reference and display threshold are set using the general FELIX menu items or icons.

Select **Preference/Reference** from the main menu. In the REFERENCE MATRIX control panel, set these parameters:

Reference Point
D1: **462**
D2: **64**

Reference Shift
D1: **4.7**
D2: **117.99**

Axis Text
D1: **D1_H1**
D2: **D2_N15**

Click **OK**.

Use the **Zoom** icon on the FELIX tool bar to zoom in on the fingerprint area.

Select **Preference/Plot Parameters**. In the PLOT PARAMETERS-BASIC control panel, enter **0.025** as the **Contour Threshold**. Click **OK**.

Finally, select **Autoscreen/Save Display and Reference** to save the reference, limits, and threshold.

These parameters will be used for display, hardcopy, and scoring of all experiments in the project.

Caution: If you make any changes to these parameters, be sure to use **Autoscreen/Save Display and Reference** to save them— otherwise the changes are lost. You can change the display threshold at any time (for example, after processing and scoring some test spectra), but the reference must be set before you select **Autoscreen/Setup Scoring** to define scoring parameters.

7. Setting other display parameters

*Many other display parameters can be changed and saved along with the project by selecting **Autoscreen/Setup Display**. These include the display parameters for the control spectrum and test spectra in contour mode and overlay mode and those for display of control peaks. For this lesson the default values are used.*

Note:

- If you have processed any test spectra, the overlay of the first available test spectrum over the control spectrum is displayed after you select **Autoscreen/Setup Display**. Otherwise, only the control spectrum is displayed.
- When you display a single spectrum, the parameters in the Control Spectrum group are used if it is the control spectrum, and the those in the Test Spectra group are used if it is a test spectrum.
- If you display an overlay of two or more spectra, the control spectrum is always used as the base spectrum and the control peaks are displayed if you select this option.
- The parameters in the Cross Peaks group are used only for displaying the control peaks. Test peaks are never displayed. If you check the **Draw Cross peak on Control** option, control peaks are displayed on the control spectrum—either in single spectrum or overlay mode.
- These parameters are saved along with the project. The next time you open the project they are automatically loaded.

8. Picking peaks and importing an assignment for the control spectrum

In the Autoscreen Experiments Table, double-click the **Control** spectrum to display it.

Activate the spectral window and select **Peaks/Pick Region** from the main menu and use the default parameters to pick all the fingerprint peaks.

About 82 peaks are picked and displayed in the Peaks-xpk:peaks table.

Tip:

- You should pick all peaks as control peaks, even if you are only interested in some of them. This guarantees better matching of control peaks to test peaks in the subsequent scoring process. If you prefer not to score all peaks, you can define those interesting peaks as ROI peaks (see Step 15 for further details).
- You should also use identical peak-picking parameters for both control peaks and test peaks to avoid artificial peak displacements. For this purpose, always select **Regular** as the **Peak Picker** in the ND PEAKPICK PARAMETERS control panel, since that is always used during peak picking of a test spectrum.

Select the **Autoscreen/Import Assignments** menu item. In the IMPORT ASSIGNMENTS control panel, select **BMBR Assignment Table** as the **Assignment File Type** and select the file **bmrb_assign.tbl** from the browser.

Click **OK**.

The output window reports that 17 peaks have been assigned. The assignments are also updated in the Peaks-xpk:peaks table.

To display the assignments on the spectrum, select **Autoscreen/Setup Display**, select **Residue** for **Peak Labels**, and click **OK**.

Caution: The bmrb_assign.tbl file is not a real or complete file and should only be used for demonstration purposes.

9. Setup of scoring parameters

In this step you set up parameters for peak picking in and scoring of test spectra.

Select **Autoscreen/Setup Scoring**. In the 2D SCORING PARAMETERS control panel, click the **Advanced** button to review parameters in the ADVANCED PARAMETERS FOR 2D SCORING control panel. Leave all the default values unchanged and click **Cancel** to return to the 2D SCORING PARAMETERS control panel. Leave its default values unchanged and click **OK**.

For an explanation of the parameters for scoring, please see Chapter 1, *Theory* in the *FELIX User Guide*.

A Peak Displacement Table is displayed. The table contains the following columns:

Table 3 Columns in the Peak Displacement Table

Column	Description
id	Numbering of the control peak in Peaks-xpk:peaks table. It is always used for identifying a control peak.
asg1	The assignment of the control peak in D1. If not assigned, value is “null”.
asg2	The assignment of the control peak in D2. If not assigned, value is “null”.
cntrib	The contribution of the peak to the total score of the experiment. It is usually calculated based on the **shift1**, **shift2**, **shape**, and **weight**.
shift1	The absolute chemical shift displacement between the matched peak pair in ppm along D1. If no matching test peak, it remains zero.
shift2	The absolute chemical shift displacement between the matched peak pair in ppm along D2. If no matching test peak, it remains zero.
shape	The similarity of the shapes of the matched peak pair; 0 = least similar, 1 = identical. If the peak shape is not used for scoring, value = 1.
weight	Weight of contribution of the peak to the total score of the experiment. An ROI (region of interest) peak has weight greater than 0. By default all peaks in the Peaks-xpk:peaks table are taken as ROI peaks when setting up scoring. You can change the weight of a peak manually from the table.
tstcen1	The chemical shift of the matching test peak in ppm along D1. If no matching test peak, it remains zero.
tstcen2	The chemical shift of the matching test peak in ppm along D2. If no matching test peak, it remains zero.

Note:

- The Peak Displacement Table is updated when you score a spectrum, when you display a histogram, or when you display the overlay of a test spectrum over a control spectrum. The ID of the current test spectrum is displayed in the title of the table.
- All peaks are taken as ROI peaks by default (i.e., weight = 1). If you are interested in only a subset of these peaks, see Step 15 for more information.

10. Processing and scoring test spectra

Once you have set up the scoring parameters, you can process and score all the test spectra.

Select **Autoscreen/Go** from the Autoscreen Experiments Table.

The two test spectra are processed and scored against the control spectrum in turn, then a histogram of scores vs. experiments is displayed. The Autoscreen Experiments Table is updated with the scores and status of the test experiments.

Note:

- For each test spectrum, a summary of the scoring results is displayed in the output window. Note the fitting of unmatched control peaks to the test spectrum and the identification of unmatched test peaks. This information is also saved in an ASCII file named as TEST_CONTROL.sco in the directory defined by the project path for ASCII files (see Step 3 for more about project paths), where TEST and CONTROL are the IDs of the test and control experiments, respectively. The contents of this file are automatically displayed in the output window when you double-click the test spectrum in the Autoscreen Experiments Table.
- Although unmatched test peaks contribute to the score of the experiments, they are not saved in the Peak Displacements Table. So double-clicking the test spectrum in the Autoscreen Experiments Table is the only way to view them in the output window.

Experiment annexinXI_34 shows a higher score than the other experiment, which usually indicates a stronger binding of the ligand to the protein.

*There are five methods for processing and/or scoring test spectra on the **Action** menu in the Autoscreen Experiments Table, which are used for different purposes. These are shown in Table 4.*

Table 4 Action Menu Processing/Scoring Methods in the Autoscreen Experiments Table

Method	Purpose
Process Selected	Process the highlighted spectrum or spectra. If the control spectrum is selected, it is ignored.
Score Selected	Process (if not processed) and score the highlighted spectrum or spectra. If the control spectrum is selected, it is ignored.
Go	For each of the test spectra, process it if not processed and score it if not yet scored.
Rescore All	Re-score all test spectra.
Reprocess/Rescore All	Re-process and re-score all test spectra.

> **Tip:** The Peak Displacement Table is not updated at this moment. To display and update it for a certain spectrum, double-click it in the Autoscreen Experiments Table.

11. Viewing clusters

*Using the **Autoscreen/View Clusters** menu item groups experiments that share common displaced peaks, providing a way to locate the residues of the protein whose chemical shifts were affected by the close contacts of the ligand in different experiments.*

Activate the spectral window and select **Autoscreen/View Cluster**, leave the default value of **Cluster Threshold** unchanged, and click **OK**.

The score matrix is displayed showing one cluster in green.

Move the crosshair cursor over the green area to display the peak number, experiment name, and contribution of that peak to that experiment. Press <Esc> when you are done.

Tip:

- The experiment numbers and peak numbers are reshuffled, so you must use the crosshair cursor (automatically displayed after selecting **Autoscreen/View Cluster**) to identify the peaks and experiments in the clusters. If you want to return to the crosshair cursor after pressing <Esc>, select **Autoscreen/View Clusters** again.
- If you want to ignore peaks that have small displacements, select **Autoscreen/View Cluster** and increase the value of **Cluster Threshold** in the VIEW CLUSTER control panel. Peaks with a contribution smaller than the **Cluster Threshold** are ignored.

12. Analyzing the scoring results

Once you have an overview of all experiments, you can investigate the interesting experiments and interesting peaks.

First highlight Experiment **annexinXI_34** (the one with the highest score) in the Autoscreen Experiments Table and click the **Peak Contribution Histogram** icon. Leave the default values in the PEAK CONTRIBUTION HISTOGRAM OPTIONS control panel and click **OK**.

A histogram of contributions vs. peaks for this experiment is displayed. The Peak Displacement Table is updated with the scoring data for Experiment annexinXI_34.

To view the peak displacements, double-click Experiment **annexinXI_34** in the Autoscreen Experiments Table.

The overlay contours of Experiment annexinXI_34 over the control spectrum are displayed, together with the displacement arrows and control peak labels. The scoring results are also summarized in the output window.

To get a clearer view of the displacement arrows, you can:

- *Select **Autoscreen/Setup Display** to change the **Peak Symbol**, **Peak Labels**, and other settings.*
- *Double-click the row of an interesting peak in the Peak Displacement Table to zoom in on that peak, or highlight several peaks in the table and click the **Zoom on Peaks** icon to zoom in on them. You can also use the **Zoom** icon on the FELIX tool bar or the <+> and <-> keys on your key pad to change the zoom ratio.*

In the Autoscreen Experiments Table, click the **Peak Contribution Histogram** icon again to display the histogram of contributions vs. peaks of Experiment **annexinXI_34**.

Peak 73 has the largest contribution and seems to be an interesting peak.

On the Peak Displacement Table, click the **Sort Contributions** icon.

The peaks are now listed in descending order of their contributions to the score.

Highlight the first row, Peak 73, and click the **Zoom on Peaks** icon or simply double-click the row of peak #73.

The display zooms in on the displacement between control peak 73 and its matching test peak.

To display the titration of Peak 73, that is, its contributions in different experiments, highlight this peak in the Peak Displacement Table and click the **Titration** icon.

A histogram of contribution vs. experiments is displayed. This also shows that this peak has a much greater displacement in Experiment annexinXI_34 than in the other experiment.

13. Manually editing scoring results

In the Autoscreen Experiments Table, highlight Experiment **annexinXI_34** and click the **Overlay** icon.

Experiment annexinXI_34 is displayed over the Control spectrum together with the displacement arrows.

Click the **Undo Sort Contributions** icon in the Peak Displacement Table, then double-click **peak 7** in the Peak Displacement Table to zoom in on the spectral area around it.

All control peaks appear to be correctly matched to the test peaks, so manually editing is not needed in this experiment. For demonstration purposes, the following operations assume that you do not like the currently matched test peaks for control peaks 7 and 8 and want to change them.

First select peaks **7** and **8** in Peaks Displacement table and click the Zoom icon so that peaks 7 and 8 are clearly visible.

To remove the current matching, select **Edit/Remove Displacement** from the Peak Displacement Table and click control peaks 7 and 8.

Press <Esc> to exit this mode.

This erases the displacement arrows.

Note: When you click <Esc> to exit a certain cursor mode, make sure that the spectral window is activated. If not, click on the title bar of the spectral window to activate it.

Now select **Edit/Change Displacement** from the Peak Displacement Table and click control peak **7**.

Keep the mouse button depressed and drag the cursor to a location that you think is the best matching test peak and release the button.

Repeat this for control peak 8.

Press <Esc> to exit this mode.

This matches peaks 7 and 8 to the desired test peaks. All the changes you've made are reported in the output window and updated in the Peak Displacement Table.

Note:

- A control peak can be matched to only one test peak. This means that changing the displacement of a control peak automatically erases the original displacement.
- A displacement you define is scored the same way as an automatically determined one, except that there are no minimum and maximum limits and the destination is not checked.

14. Exporting scoring results

Activate the spectral window and select **Autoscreen/Export Score**. In the EXPORT SCORES control panel, set **Contents** to **All Scores** and **Delimiter** to **Tab**. Enter the filename **Test2** as **Filename** (a .txt suffix will be added automatically).

Click **OK**.

All the experiments and their scores are listed in the Test2.txt file.

Repeat the previous box with **Contents** set to **All Scores Sorted**.

This lists all experiments and scores in descending order.

Repeat the first box of this step with **Contents** set to **Scores and Titration**, **Number of Experiments** set to **2**, and **Number of Peaks** set to **10**.

The scores of the top two experiments, in descending order of scores, and the contributions of the top 10 peaks that have the greatest sum of contributions to the two experiments are reported.

Note: This function is intended to give a summary of the "interesting peaks in the interesting experiments." You can choose the numbers of experiments and peaks to report.

<Shift>-click to select the two test experiments in the Autoscreen Experiments Table and <Ctrl>-click to select peaks 60, 63, and 73 in the Peak Displacements Table. Then repeat the first box of this step with **Contents** set to **Titration Selected**, **Delimiter** set to **Tab**, and **Use Comments as Concentration** toggled **off**.

*The contributions of the selected peaks in the selected experiments are reported. Such a report is intended for calculation of K_d based on titration. If you have specified the concentration of the experiments in the comment column in the Autoscreen Experiments Table, you can toggle on **Use Comments as Concentration** to include that information in the report.*

Repeat the first box of this step with **Contents** set to **C2 QSAR Table**.

All experiments and scores are listed in a format suited for QSAR study with the Cerius^2 program.

To import Autoscreen results into Cerius^2 for QSAR study, take the following steps:

1. Start Cerius2. Select the QSAR deck and click the **Show Study Table** item on the QSAR card. This brings up a new, empty QSAR Study Table.
2. In the QSAR Study Table, select **File/Import...** In the Import/Table control panel, uncheck **File Contains Row Labels**, check **File Contains Column Labels**, select the file-name from the list box, and click **Import**. The experiments and score are displayed in the Table Manager.

15. Scoring again with ROIs (regions of interest)

After getting an overview of all peaks in all experiments, you may want to focus on some interesting peaks in some interesting spectra instead of looking at all of them. When setting up the scoring parameters (see Step 9), all peaks in the Peaks-xpk:peaks table are taken as ROI peaks with weight equal to 1.0 by default. The following tasks demonstrate some of the methods for defining a subset of the peaks as ROI peaks.

Highlight the calcyclin spectrum in the Autoscreen Experiments Table and click the **Draw** icon to display it.

If the peak labels and peak numbers are not displayed, select **Autoscreen/Setup Display** from the main menu and choose **Small Cross** for **Crosspeak Symbol** and **Number #** for **Peak Labels**.

click **OK**.

Select **Autoscreen/Define Region of Interest/Remove All** to remove all peaks from the ROI set.

This sets all peaks as nonROI peaks with weight equal to 0. Note the change of their color in the spectrum window.

Select **Autoscreen/Define Region of Interest/Add Region**. Drag out a rectangle around the peaks with H^1 chemical shift greater than 9.0 ppm.

Note the change of color of these peaks and the report in the status bar:

```
Displaying 9 ROI peaks. Total 82 peaks.
```

In the Peak Displacement Table only these ROI peaks have non-zero weights.

Select **Add One Peak**. Click Peaks 1–5 and 73. Then press <Esc> to quit.

You have now about 15 ROI peaks. See the status bar again for the number of ROI peaks.

After defining ROI, you can rescore the spectra you are interested in. Highlight Experiment **annexinXI_34** in the Autoscreen Experiment Table and select **Action/Score Selected Spectra**.

Its score is reduced to 2.285. Only the ROI peaks, displayed in yellow by default, show displacement arrows in the spectrum window. Both the Peak Displacement Table and the output window show the scoring contributions of the ROI peaks only.

Click the **Peak Contribution Histogram** icon with Experiment annexinXI_34 still highlighted, to see the histogram of the contributions of the ROI peaks. You can select either **Peak IDs** or **Residue Numbers** as the x coordinates.

To score all experiments based on the newly defined ROI peaks, select **Action/Re-score All Spectra** and click **OK** in the control panel.

Caution:

- Only peaks from the original Peaks-xpk:peaks table can be defined as ROI or non-ROI peaks. If you want to add new peaks, you have to do peak picking with the **Peaks** menu and set up scoring parameters again with **Autoscreen/Setup Scoring**.
- When you set up scoring again, you lose all scoring results if you have changed the Peaks Table.
- If you zoom the spectra or resize the spectra window, the color of ROI peaks is lost. You can select **Autoscreen/Define Region of Interest/Draw ROI** to restore their color. You can define the color of ROI peaks with the **Autoscreen/Setup Display** menu item.

16. Printing spectra and histograms

To set up for printing, select **File/Print Setup** from the main menu. For example you may want to set Orientation as Landscape.

To get a print preview, select **File/Print Preview**.

To print either a spectral display, a histogram, or a table, select **File/Print**.

17. Displaying molecules and scores in Insight II

To display experiments associated with a molecule, first start Insight II on a UNIX machine and navigate to the NMR_Refine module.

Next copy the **demo.pdb** file to the UNIX directory where you started InsightII. Select **Molecule/Get**, and choose **PDB** as **Get File Type**. Click **Execute**.

The protein molecule is displayed in InsightII.

Highlight the annexinXI_34 experiment in the Autoscreen Experiments Table. Select **Action/Color Scores** from the table.

In the COLOR RESIDUES BASED ON SCORE control panel, select **InsightII** as the **Format** and enter **color** as the filename.

Four assigned peaks with non-zero contributions are exported into file color.tab.

Copy the **color.tab** file to the UNIX directory where you started InsightII.

In Insight II, select **Query/Color_By_SAR_Score**. In the control panel, set these parameters:

Sar_Molecular_Name	**DEMO**
SAR_Score_Filename	**color.tab**
Neutral_Color	**white**
Num_Intervals	**10**
Low_Score	**0.02**
High_Score	**0.2**

Click **Execute**.

Tip: If you are using Insight II version 980 or older, in which the **Query/Color_by_Sar_Score** command is not available, you can select **File/Source_File** to open the **color_by_score.bcl** script file to set up this command. The script file resides in the sar directory.

Wait until the rendering of the Connolly solid surface is complete. The residues that contribute to scores (between 0.02 and 0.2) are displayed in red. This may take several minutes.

18. Exiting FELIX

To exit FELIX, select **File/Exit**.

Note:

- In real-world practice, you may want to start a new project at this point. To do so, select **File/Save** to save the current database, then select **File/New** to start a new database. Next follow Step 3 to start a new project.
- If you exit FELIX before finishing a project, be sure to save the database when you exit (it is not important to save the session). In the new session you can open the saved database and then select **Autoscreen/Project** to load the project.

Lesson 2: Processing and Visualizing Multiple 1D spectra

This lesson presents the basic steps of analyzing multiple 1D spectra using the Autoscreen module of FELIX.

The topics covered in this lesson are:

- Setting up and adding experiments to a project.
- Processing a control experiment.
- Processing multiple 1D experiments.
- Using the overlay tools to visualize the results.

1. Setting up for the lesson

If not done yet, set up the tutorial files as described in "Setting up tutorial files" in the preface, *How To Use This Book*.

The files for this lesson are located in the **Autoscreen\Lesson2** folder.

The files are briefly described below:

Table 5 Files in the sar directory

File	Purpose
1*	fid and parameter files of control experiment
2*	fid and parameter files of first test experiment.
3*	fid and parameter files of second test experiment.
4*	fid and parameter files of third test experiment.
exps.txt	A list of experiments, used to add experiments to the Autoscreen project.

The exps.txt file is a list of input experiments and their associated structural files, with each line specifying the experiment ID, fid file (with relative path; see Step 3 for project paths), file type, structural filename (optional), and comments (optional):

```
control     1/fid  bruker_fid

testa_1     2/fid  bruker_fid

testa_2     3/fid  bruker_fid

testa_3     4/fid  bruker_fid
```

2. Starting FELIX

Start FELIX by double-clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

In the OPEN DATABASE FILE dialog box, navigate to your working directory which contains the lesson 1 tutorial files.

If you used the default suggestion, this will be: **C:\Felix_Practice\Autoscreen\Lesson2**.

Enter **test** as the filename and click **O**K to build a new database file.

Note: When FELIX starts you may need to adjust the size of the text window by clicking on its upper border and dragging it down a bit to see more of the upper spectrum display frame.

3. Setting up the project

Select the **Autoscreen/Project** menu item from the FELIX menu bar. When you see the AUTOSCREEN PROJECT control panel, leave the **Project Name** unchanged (**sar**), change the **Project Type** to **1D** and click **OK**.

You should see this in the status bar:

```
Created new Autoscreen project 'sar'.
```

Note: Currently a project name is limited to less than nine lower-case alphanumeric characters. You can create only one Autoscreen project in a database file. Once a project is finished, you can select the **File/New** menu item to open a new database after saving the current one, and then repeat this step to create a new project.

The VERIFY DIRECTORIES control panel appears next. It allows you to verify some important paths used to access or save the following files:

Raw spectrometer data files

ASCII files, including file list

Processed 1D files (.dat files)

For this example, you are using relative paths for all experiments and molecular files, so it is important to verify the project paths.

Make sure that the paths in the VERIFY DIRECTORIES control panel are similar to the following:

Spectrometer Data:
C:\Felix_Practice\Autoscreen\Lesson1

ASCII Text Files:
C:\Felix_Practice\Autoscreen\Lesson1

Processed 1D Files:
C:\Felix_Practice\Autoscreen\Lesson1

Click **Browse** next to any of the paths to select a directory interactively or you can directly enter a directory name.

Click **OK**.

Note:

- If you use the **Browse** button to interactively select a directory you move to the directory that contains the files of that type. No specific **Filename** selection is required. For example if you were interactively selecting the **Spectrometer Data** directory you would move to the directory that contains the experiment numbers. Once in this directory you would see the various experiment numbers (1, 2, 3, 4).
- A new window containing an empty Experiments table is open and displayed to the left of the spectral window. By default, whenever a new window (table or spectral) is open, FELIX automatically re-arranges the layout of the windows. You can turn off this feature by selecting **Preference/Frame Layout** from the main menu and set **Action** to **None**. You can also do the automatic re-arrangement at anytime by selecting **Window/Auto Arrange**.
- When one or more table windows are open, only the menu and tool bar of the currently activated window are visible. If you want to select a certain menu item or tool bar icon, be sure to click the corresponding window first to activate its menu and tool bar (if any).

Table 6 Description of items in the Autoscreen Experiments Table

Column	Description
Id	ID of the experiment.
Score	Total score of the experiment. (Not used for 1D spectra.)
Thresh	Threshold used for peak picking if scored. (Not used for 1D spectra.)
Status	Status of the experiment, with 0 standing for nonprocessed, 1 for processed, and 9 for control spectrum.
Fid	File name of the FID file, if any.
Type	File type of the FID file, fid for Varian FID file, bruker_fid for 1D Bruker file.
Struc	Filename of a molecule, with extension .pdb standing for PDB file, .car for CAR file, and .mol for MDL file. It can also be filename of a list of molecules if another extension is used. (optional)
Comment	Comments (optional)

> **Tip:** If you want to change the project paths again, use the **Edit/Verify Directories** menu item from the table or the **Autoscreen/Experiment/Verify Directories** item on the main menu bar.

4. Adding experiments to a project

The following steps demonstrate three ways of adding experiments to an Autoscreen project.

Option 1-Add one experiment at a time

Activate the spectral window (click inside **Frame 1** or on its top border) and select the **Autoscreen/Experiment/Add One** menu item.

In the ADD ONE EXPERIMENT control panel, select **Bruker (fid)** as the **Spectrum File Type**, enter **Control** as the **Experiment ID**, and fill in the **Comment** with `This is the control experiment`.

Double-click on directory **1**, select the **fid** file, and click **OK** to add this experiment to the project.

The Autoscreen Experiments Table is updated with the newly added experiment.

The ADD ONE EXPERIMENT control panel is displayed again for you to add another experiment.

Select **Cancel** to close it.

Option 2-Add experiments from all files in a directory

First clean up from your test of the previous option:

Highlight the row of interest (or all rows if you added more than one experiment) in the Autoscreen Experiments Table and select the **Edit/Delete Experiment**s menu item to remove the experiment.

Note: When you highlight the experiment table, the FELIX menu bar and icons are changed to reflect the options that are appropriate for use with that particular table.

To go back to the default menu bar, click inside **Frame 1** or on its top border.

Next, add experiments from all files:

Activate the spectral window and select **Autoscreen/ Experiment/Add All Files**. In the ADD ALL EXPERIMENTS control panel, change **To Dir** to **4**, the highest experiment number. Then click **OK**.

All four experiments are added to the project, with the first experiment taken as the control spectrum.

Tip: This function expects numbered Bruker experiments. If the experiments are not consecutively numbered, you can instruct FELIX to skip one or more between every two experiments, or it will automatically ignore nonexistent experiments.

Option 3-Add all experiments listed in a file

First clean up from your test of the previous option:

Highlight all rows in the Autoscreen Experiments Table. From the table, select the **Edit/Delete Experiments** menu item to remove all the experiments.

Next, add experiments by reading in a list from a file:

Activate the spectral window and select the **Autoscreen/ Experiment/Add From File List** menu item.

In the ADD EXPERIMENTS FROM FILE LIST control panel, select the file **exps.txt** and click **OK**.

This adds four experiments to the project, using the experiment list in the exps.txt file. These experiments are the ones that we will use in subsequent processing and scoring in this lesson.

5. Processing a control spectrum

Unless you are using processed data, one of the most important steps in using Autoscreen is processing the control spectrum. The processing parameters used during this procedure are used for the subsequent processing of all other test experiments.

Highlight the first experiment in the Autoscreen Experiments Table and select **Action/Process Control Spectrum** from the menu bar of the table.

Next you are guided through the processing of this 1D experiment. The procedure is similar to standard 1D processing in FELIX (when using the Process/1D Data Processing command).

Keep the default parameters unchanged and click **OK** in the 1D HEADER control panel.

The EZ 1D DATA PROCESSING control panel appears next.

Unselect both the **Window Function** option and the **Phase Correct Option**. Leave only the **FT** option selected and click **Apply**.

The spectrum is Fourier transformed and the EZ 1D Data Processing menu remains on the screen so you can try other processing combinations. You may need to adjust the position of the menu so that it doesn't obscure the spectrum.

Next, phase the spectrum.

Turn the **Phase Correct** option **ON** and set the **Method** to **Real Time**. Click **Apply**.

The spectrum is Fourier transformed. The phasing menu comes up.

Click the **Pivot** button. Using the cursor, click on the large peak near the center of the spectrum.

Next, using the **zero order phasing** slider adjust the large peak in the center until it is in phase. Then using the **1st order phasing** slider adjust the right-most peak until it is in phase.

Note: You may have to click the **Coarse** button to get more range for the 1st order phase adjustment.

When the spectrum is in phase, click **OK**.

The phased spectrum appears and the processing menu is displayed again.

Change the **Phase Correct Method** to **Use Current**. Click **Apply**.

If you have properly setup the phasing parameters the spectrum will be transformed and phased properly at this point.

Note: The spectrum is still a bit noisy.

Now, apodize the spectrum, which demonstrates the interactive adjustment method.

Turn the **Window Function** parameter **ON** and set the **Method** to **Sinebell squared**. Click **Apply**.

When the Sinebell parameters menu displays, enter a **Phase Shift** value of **60.0** degrees and click **OK**.

*When you click **OK** the EZ 1D Data Processing tool executes the processing choices you made. In this case it apodizes, transforms and phase corrects the spectrum. For many of the data processing choices you want to be able to try many different parameter combinations and visualize the result. The Interactive Processing choices allow you to interactively adjust most of the various processing parameters.*

In the **Interactive Processing** group of buttons click on the **Window** button to interactively adjust the apodization parameters.

A window displays with sliders to allow you to adjust the number of points to apodize over and the phase shift.

For the **Display Option** select **Digital FT**.

This is the appropriate type of FT to use for this Bruker digitally oversampled data.

At this point the apodized fid, the apodization function and the transformed spectrum all appear on the display.

Experiment with various combinations of **Window Size** and **Phase Shift** and note the results on the transformed data.

You may also enter values directly in the parameter fields. Try a **Window Size** of around 512 points and a **Phase Shift** of about 70.0 degrees.

Click **Keep** when you are done adjusting the data.

In a real task, you would continue to adjust the processing parameter until you get a combination you like. At this point in the tutorial, finish the processing of the Control spectrum.

The EZ 1D Data processing menu displays again.

Click **Apply**. Verify that the correct apodization values are entered (512 and 70.0) and click **OK**.

The EZ 1D Data processing menu displays again.

Click **OK**.

This indicates that you are done with the processing and want to accept the parameters.

The Reference 1D Data menu appears.

Click the **Cursor** button and select the right-most peak.

In the **Reference** menu enter a value of **0.0** for the **Reference PPM** value and click **OK**.

6. Processing all spectra automatically

Highlight the Autoscreen Experiments table by clicking on its top menu border. Then select the **Action/Process/ Reprocess All** command from the menu bar.

You will see FELIX run through the processing of all the 1D spectra using the processing parameters you entered.

Next, demonstrate some of the visualization options for 1D spectra.

7. Viewing and analyzing the 1D spectra

Click on the first row in the Autoscreen Experiments table.

You'll notice that this makes sure that the table is selected and the appropriate Autoscreen Experiment table menu items and icons are displayed.

Click the **Draw** icon. This displays the first spectrum in the table. Try using the **Draw Next** and **Draw Previous** icons. These icons scan through the various spectra and cause the **next/previous** spectrum to be displayed.

There are also various tools used to compare spectra.

Now use the overlay tool to compare a given spectra with the control spectrum.

Select the second row in the table by clicking on its item number. That row will highlight. Then click the **Overlay** icon. The control spectrum (row 1) is shown together with the selected spectrum. You may use the **Overlay Next** and **Overlay Previous** icons to compare other spectra with the control spectrum.

Note: The above example illustrates how to compare the control spectra (the spectrum used to define the processing parameters) with the other spectra. However, in many cases you need a more versatile tool to compare multiple spectra at one time. Use the **Overlay Multiple** icon to compare many different spectra at one time.

Select all rows in the experiment table: click on the first row item number and hold and drag the mouse down to the fourth row item number. Or, click on the row one item number then release the mouse. Then hold the shift key down as you click on the row four item number. Either of these methods should select all rows in the table.

Click on the **Overlay Multiple** icon.

*The selected spectra appear on the display along with the **1D Overlay Setup** menu. This menu allows you to select how many spectral combination to show on the screen. Each of these displayed spectra can be either an original spectrum or a subtractive combination of any of the spectra you selected from the Autoscreen Experiments table.*

*The **Number of Spectra to Display** parameter represents the number of these combinations which you wish to display at one time. The **Spectrum Overlap** parameter allows you to determine the extent of overlap. A "0.0" represents no overlap. A "1.0" represents total overlap.*

Set the **Number of Spectra to Display** parameter to **3**. This causes the first three rows to highlight.

For spectrum 1 leave the **Method** on **A** and the **Spectrum A** parameter as **control.dat**.

For spectrum 2 set the **Method** parameter to **A-B**, indicating that you want this second spectrum to be a subtraction. Set the **Spectrum A** to **control.dat**. Set **Spectrum B** to **testa_1.dat** which is the first test spectra.

For spectrum 3 leave the **Method** set to **A** and set the **Spectrum A** parameter to **testa_1.dat**.

Click **Apply** to see these three spectra.

You'll see that three spectra are displayed. The first is the control spectrum. The second is the control spectrum minus the first test spectra. The last spectrum in this group is the first test spectra.

Now, manipulate the data to make finer comparisons.

*You'll notice that in row two of the **1D Overlay Setup** menu that the **Adjust** button is not grayed out. Since this is a subtractive combination you can adjust the relative shift between the two spectra and the relative intensity of each one.*

In row 2 of the **1D Overlay Setup** menu, click **Adjust**.

A real-time adjustment menu appears where you can control the intensity of each of the two spectra being subtracted along with the relative shift in points between the two.

Note: Initially the intensity of the first spectrum is "1.0" and the intensity of the second spectrum is "-1.0". Since these two spectra are very similar the subtracted spectrum is mostly noise.

Try adjusting the shift between the two spectra and note the effect on the subtraction spectra in the center. You may also try adjusting the intensity of either of the two spectra which make up the subtraction.

To exit this routine click **Quit** to exit the real-time adjustment menu and then click **Cancel** to exit the **1D Overlay Setup** menu.

This concludes the Autoscreen Lesson 2 tutorial on processing and visualizing 1D data.

8. Exiting FELIX

To exit FELIX select **Frame 1** and select **File/Exit** from the menu bar. Unselect the options to **Save Current Session** and **Save Current Database**.

Click **OK** to exit the program.

6 Using the Database

About the lesson

In this tutorial you will learn about the commands and functionalities of the database commands within FELIX.

Topics

- Database file building, opening, and viewing.
- Entity viewing, loading, and storing.
- Schema modification.

Lesson 1: Introduction to the database utilities

1. Starting FELIX

Start FELIX by double clicking the **Felix** icon on your desktop, or by clicking the **Start** button on the Windows taskbar, then selecting **Programs/Accelrys Felix 2002/Felix 2002**.

If FELIX prompts you to restore from last session, click **Cancel**.

FELIX displays an OPEN DATABASE FILE dialog box. Here you will create a database file.

In the OPEN DATABASE FILE dialog, navigate to a directory where you want to be the working directory.

Then, type in a new filename (e.g. **dba0**) to open a new empty database.

FELIX displays the name of the database file (as well as the directory of the file) in the title bar of the FELIX main window, confirming its selection:

The FELIX database has a hierarchical structure which may be represented as in Figure 2.

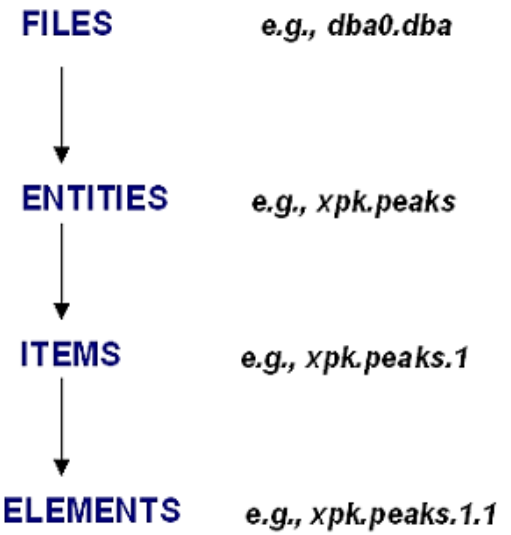

Figure 2

Files are composed of entities or tables, whose structures are defined using schema files. The entities are in turn composed of items, which are themselves an arrangement of elements. Begin familiarizing yourself with the dba file system by working through some of the dba utilities, as in this lesson.

2. Closing the current dba file and creating a new one

Click inside the command text field at the bottom of the FELIX window. Enter the following commands (press <Enter> after typing each command), which close the current dba file, create a new one and open it, and then list the entities in the new dba file:

```
dba file close quit

dba file build test
```
(if you already have a dba file named test, use some other name)

```
dba file open test

dba file show
```

You should now see the following in the output window:

```
item page name schema
---- ---- ---- ------
1 1 dba dba
```

which indicates that only the default (empty) database file exists.

Note: FELIX remembers up to 10 used commands. You can use the up or down arrow keys to browse through the last commands and edit them.

3. Generating 2D cross peaks

Select **File/Open** to open any 2D spectrum (e.g. the one created in Tutorial 2 (*Lesson 1: 2D processing, display, and analysis*). Select **Peaks/Pick Region** to pick a small region of peaks.

FELIX uses the appropriate entity (i.e., xpk.sch) and automatically renders the data.

4. Checking the database file structure and seeing how it has changed

Now enter:

dba file show

You should now see the following in the output window:

```
item page name schema
---- ----- ---- ------
1 1 dba dba
2 3 xpk dba
```

5. Viewing the contents of the entity xpk

Now enter:

dba entity show xpk

You should see this in the output window:

```
item page name schema
---- ----- ---- ------
1 5 peaks xpk
```

6. Inquiring about particular items and elements of the entity xpk:peaks

Enter:

dba entity show xpk:peaks

FELIX displays a list of the cross peak footprints.

You can also specify the display of a particular item in an entity.

Enter:

```
dba item show xpk:peaks.2
```

Information similar to this appears in the output window:

```
2 216.186 2.383 0 null 721.749 3.269 0 null
```

Next you specify a particular element in an item.

Enter:

```
dba element show xpk:peaks.2.2
```

The following information is displayed:

```
216.186
```

Since the element fields are named, you can use an equivalent command:

Enter:

```
dba element show xpk:peaks.2.cen1
```

The same information is displayed as for the preceding command:

```
216.186
```

The other database utilities have the same syntactic logic.

7. Loading the value of an element

Now you load a value from the database using the name check.

Enter these commands:

```
dba element load xpk:peaks.2.cen1 check

lis check
```

The following value is displayed:

```
216.186
```

You can change the value of any element in the database using a identifying number.

Enter:

```
dba element store xpk:peaks.2.cen1 104.8
```

You can also change the value of any element in the database by using names instead of numbers.

Enter these commands:

```
def check 104.8

dba element store xpk:peaks.2.cen1 &check
```

8. Modifying schema

Begin by copying one of the existing schema, ***asg.sch****, to a new schema with the name* ***wrd.sch****. Then edit the new schema file*

Note: To locate the FELIX schema files, locate the database folder in the path where the FELIX 2002 executable is located.

By default this path is

C:\Program Files\Accelrys\Felix 2002\database.

Open the database folder. Locate and open the folder called schema. Find the schema file asg.sch.

Copy the existing schema, *asg.sch*, to a new file named *wrd.sch* in your working directory using Windows Explorer. Next use Notepad to edit the new wrd.sch schema file to the following:

```
>  c**wrd.sch
>  wrd
>  5item       i k    01   06   (1x,i5)
>  cenpnt      r k    01   10   (f10.3)
>  wid         r      01   10   (f10.3)
>  cenppm      r k    01   10   (f10.3)
>  name        c      32   34   (2x,a32)
```

9. Passing data to and getting data from a new database entity

Generate a new entity by entering this command:

```
dba entity build weird wrd 1
```

*This generates a new entity with the name **weird**, which is based upon the schema **wrd.sch**, using a single occurrence of that schema.*

Store a value in an element of your new entity by entering:

```
dba element store weird.1.5 abcdef
```

or

```
dba element store weird.1.name abcdef
```

Both commands specify storing the string in the entity named weird, item 1, element 5 (again note that the element fields are named).

Give that element a name by entering:

```
dba element load weird.1.name var
```

Confirm that the element has been named by entering:

```
lis var
```

You should see the following:

```
abcdef
```

Write the entity to an ASCII file by entering:

```
dba entity write weird weird.txt
```

Now read the ASCII file back in as another entity by entering:

```
dba entity read test weird.txt
```

Confirm the success of this set of operations by entering:

```
dba element load test.1.5 newvar
```

```
lis newvar
```

The following should be displayed:

```
abcdef
```

*To view any entity via tables you can use the **Edit/Table** command. This opens a control panel from which you can choose the entity that you would like to display in a table.*

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