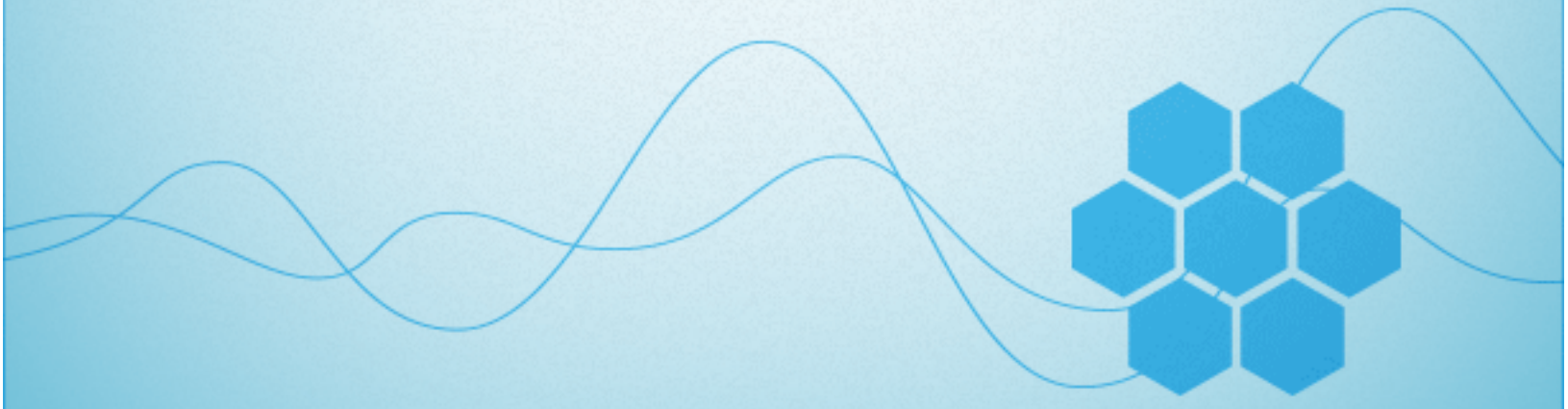
CHENOMX

nmr suite 8.1



Chenomx NMR Suite 8.1 Tutorial

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Chapter 1. Processor

1.1 Introduction

You can use the Processor module to **process** and **calibrate** your spectra so that they can then be used in Profiler, Spin Simulator or Compound Builder.

When processing a spectrum, you'll clean up its lineshape so that it is easier to work with and easier to calibrate. You do this by applying the various processing effects described below.

When calibrating a spectrum, you'll try to accurately identify, quantify, and fit its CSI (Chemical Shape Indicator) peak. Profiler, Spin Simulator and Compound Builder use the CSI peak to determine lineshapes for any fitting or simulations that you do, so it is very important to calibrate the CSI properly.

There are a variety of processing effects available in Processor, and it is possible to apply them in any order that you prefer. However, for best results we recommend that you use them in the following order:

1. Line Broadening (if necessary)

   Line broadening is available, but many ^{1}H NMR spectra will not need it. If you have CSI linewidths of 1 to 1.5 Hz or higher, do not apply line broadening.

2. Phase Correction

   Unless you need to reverse the spectrum, phase correction should always be your first processing step.

3. Baseline Correction (2-point linear or cubic splines)

   Even if your spectrum appears to have a fairly flat baseline, consider applying a simple baseline correction layer to ensure that the spectrum baseline aligns with all compound signatures.

4. Shim Correction (if necessary)

   You will not need to apply shim correction to every spectrum, but when you do, make sure that you have properly adjusted the phase correction and baseline correction first.

5. Region Deletion

   Variation in the performance of solvent suppression pulse sequences can produce water peaks that are still much larger than all the other signals in a spectrum. Using Region Deletion to remove the water peak can greatly reduce the amount of zooming in you will need to do while profiling that spectrum.

1.2 Processing a Spectrum

Getting Started

1. Open Processor.
2. Open the spectrum `Sample.fid\fid`.

Note: the files mentioned in this chapter are part of the sample file set that came packaged with this document. They can be found in the `Sample Files\Processor` folder.

3. In the Import Agilent Raw FID Dialog:

   a. Indicate that DSS is present in this spectrum as the CSI (Chemical Shape Indicator), and set its concentration to 0.484 mM.

      Setting the correct CSI concentration ensures that quantification in Profiler is as accurate as possible.

      When you import your own spectra, use a CSI type and concentration appropriate to your samples.

   b. We will determine an accurate pH in a later step. For now, clear the pH indicators check box, and leave the pH set to 7.0, with an uncertainty (+/-) of 0.50.

   c. Clear the **Phase Correction** check box.

      For this tutorial, we will do manual phase correction.

   d. Clear the **Auto Baseline Correction** check box.

      For this tutorial, we will manually correct the baseline.

   e. Clear the **Automatic Shim Correction** check box.

      For this tutorial, we will manually apply shim correction.

   f. Clear the **Line Broadening** check box.

      Unless your CSI linewidths are significantly less than 1 Hz, you do not need to add line broadening.

   g. Clear the **Delete Water Region** check box.

      For this tutorial, we would like to see what the spectrum looks like before the water region is deleted.

   h. Set **Zero Fill** to Automatic, if it is not set already.

      This value does not normally need to be changed.

   i. Click the **OK** button.

      Your newly imported spectrum will now be visible in Processor.

Adjusting the Spectrum Phase Correction

4. Click on the **Phase Correction** button.

5. Click the **Auto** button.

   A good first step to manual phase correction is to apply the automatic phase correction, which may compute the optimal phase values for your spectrum. Even when it does not, it often still provides a reasonable starting point for further corrections. For the best results, visually inspect the automatic phase values and refine them as needed.

6. Select the **Fine Tune** checkbox to make small adjustments to the phase correction. Drag the sliders for zero-order and first-order phase angles until most peaks in the spectrum appear symmetrical.

The water peak often does not phase properly with the rest of the spectrum, so focus on other signals on the outside edges of the spectrum instead. The ideal phase angles for this sample spectrum are approximately 54° (zero-order) and -2° (first-order).

7. Click the **Accept** button to apply your phasing changes to the spectrum and exit Phase Correction mode.

Applying Baseline Correction

8. Click on the **Baseline Correction** button.
9. Click the **Auto Linear** button to have Processor locate the spectrum endpoints automatically. If you zoom in very close to the spectrum baseline before doing this, it will be easier to see how the proposed (blue) baseline changes.

   When a spectrum's baseline is already mostly flat, the automatic linear baseline correction may be all that is needed.
10. Click the **Auto Spline** button to have Processor propose a more complex baseline built out of cubic splines and breakpoints.
11. Click in the spectrum graph to add more breakpoints to the cubic spline baseline fit. Use more points where the baseline is strongly curved, and fewer points where it is more linear.

    Breakpoints normally appear on the spectrum line. Hold down the **Control** or **Command** key while clicking to place breakpoints at arbitrary positions.
12. Continue placing and moving breakpoints until the proposed baseline (the blue line) follows the spectrum baseline as closely as possible.
13. When you are satisfied with your proposed baseline, click the **Accept** button to apply your changes.

Calibrating the CSI

14. Click on the **Calibrate CSI** button.
15. Zoom in on the single DSS peak at the far right side of the spectrum. Use the **Automatic Vertical Zoom** button to ensure the best view of the single peak.
16. Click the **Calibrate Automatically** button to have Processor attempt to automatically match the red calibration peak with its counterpart in the sample spectrum.

    If your spectrum has poor phasing or baseline correction, calibrating the CSI peak automatically will not work; correct the phase correction and baseline correction before proceeding.
17. If necessary, use the mouse, adjustment handles, width controls or keyboard arrows to adjust the shape of the calibration peak to match the black spectrum line as closely as possible.

    Accurately fitting the CSI peak is very important. The CSI parameters directly affect the quality of analysis that is possible with your spectra. Perhaps most obviously, the CSI peak position calibrates the chemical shift scale. Also, the CSI concentration and peak height both factor into quantification accuracy, while an accurate CSI peak width allows you to better fit regions of the spectrum in which multiple clusters overlap.
18. When you are satisfied with your CSI peak calibration, click the **Accept** button to apply your changes.

Applying Shim Correction

19. Click on the **Shim Correction** button.
20. Shim correction corrects your spectrum for some types of systematic errors that may have occurred during acquisition. It may not be appropriate for all spectra.
21. Leave the Target Linewidth at its initial value of around 0.6 Hz.
22. Select the **Use DSS/TSP Satellites** check box.

    This option adds the ^{29}Si satellite peaks onto either side of the DSS methyl peak in the reference peak shape.
23. For some spectra, you may need to manually adjust the position of the blue reference peak to line up with the target peak in spectrum. For this sample, however, the automatic position is correct and does not need to be changed.
24. Click the **Accept** button to apply shim correction to the spectrum.
25. Click on the **Calibrate CSI** button again.

    The CSI peak needs recalibration following shim correction, since peak shapes have changed throughout the spectrum. It is important to double check the fit of the CSI peak any time you change the processing of a spectrum.
26. Click the **Find Automatically** button.
27. Click the **Accept** button.

Determining the Spectrum pH

28. Click on the **Calibrate pH** button.

    Setting the spectrum pH is very important! Most of the Chenomx Reference Compounds are pH sensitive, which means that their starting positions and transform windows in Profiler are affected by the spectrum pH.

    If you choose a spectrum pH that differs a lot from the actual sample pH, fitting peaks in Profiler becomes more difficult. Clusters will start farther than necessary from their real positions, and the transform windows may not allow you to move each cluster far enough to compensate for this.

    When you are not able to set the spectrum pH accurately, you can account for this by increasing the pH uncertainty value. This is the +/- value to the right of the pH. A larger pH uncertainty will generally lead to larger transform windows in Profiler.
29. Click the **Find Automatically** button to have Processor attempt to determine the spectrum pH automatically.

    Since you have already corrected the phasing and baseline of the spectrum, automatic pH determination will work.

    The calculated pH is 8.37.

Determining the Spectrum pH Manually

30. You can also determine the pH manually.

    Click and drag the pH slider bar to adjust the pH, or manually enter a new pH value in the pH text entry box.

    The blue, green and yellow lines, indicate the positions of the imidazole, creatinine and DFTMP peaks respectively. These lines move as you adjust the pH. You can

determine an approximate pH value simply by adjusting these lines as close as possible to the corresponding peaks in your spectrum.

31. After adjusting the pH, look at the information box to the left of the pH slider bar, and note which pH indicator compound is most active at your chosen pH.

    For spectra where the pH is near the transition between creatinine and imidazole (around 5.5), then both indicators are active.

    In this example, the active indicator is imidazole.

32. Zoom in on the two peaks for imidazole. Click the **Automatic Vertical Zoom** button to ensure the best view of the two peaks.

    The range of pH available on the slider bar changes as you zoom in, in order to yield a finer-grained control over your pH adjustments. To make the entire range of 0-14 available again, either zoom back out, or clear the **Fine Tune** check box.

33. Again, click and drag the pH slider bar to adjust the pH, or manually enter a pH value in the pH text entry box.

    As before, the indicator lines move as you adjust the pH. To obtain a more refined pH value, ensure that each line is still as close as possible to the centers of its associated peak, keeping in mind that every change in pH can move both peaks.

    As before, the measured pH should be around 8.37.

34. Click the **Accept** button to apply this change.

Now that you have finished calibrating and processing your spectrum in Processor, you can:

- Move the spectrum directly to Profiler for further analysis (using the **Jump to Profiler** button)
- Save the spectrum for later analysis in Profiler, for creating a simulation in Spin Simulator, or for creating a compound signature in Compound Builder.

Chapter 2. Profiler

2.1 Introduction

The Profiler module is the primary tool in Chenomx NMR Suite for profiling sample solutions. You can profile samples by matching peak frequencies and heights contained in a compound library with frequencies and heights in the sample spectrum. To help you become familiar with the profiling process, the first part of the following tutorial provides a step-by-step guide to fitting a sample spectrum containing only twelve compounds, none of which have overlapping signatures. The second part provides some techniques for fitting spectra in which some compound signatures overlap.

2.2 Basic Profiling Techniques

The simplest way to start analyzing a spectrum is to profile easily identifiable compounds first, as they often have additional peaks in the busier regions of the spectrum. Profiling them first can help rule out other compounds later in the profiling process. In this simple spectrum, all of the compounds are quite well separated from each other, and should be relatively simple to profile.

Single Peak Compound - Acetate

1. Open Profiler.
2. Open the sample file called `Basic Start.cnx`.

   Note: the files mentioned in this chapter are part of the sample file set that came packaged with this document. They can be found in the `Sample Files\Profiler` folder.
3. Zoom in on the single peak near 1.90 ppm.
4. Right-click on the peak, and click **Search for Compounds Near 1.91 ppm**.
5. In the Compound Table, select the compound with the highest Maximum Concentration (acetate), as this is the most likely candidate for fitting the peak.

   You may want to sort the Compound Table by Maximum Concentration to make finding the highest Maximum Concentration simpler. Click the header of the Maximum column to sort by that column. Additional clicks on the header reverse the sort order.
6. Click **Compound > Fit Automatically**, or press the space bar.

   Profiler's automatic fit suggests concentrations and cluster positions for the currently selected compound. Although it will often arrive at acceptable values, you should always review the results of an automatic fit before moving on to another compound.
7. Adjust the acetate signature (blue) as needed to match it to the corresponding peak in the spectrum (black).

   You can click and drag the controls on the horizontal and vertical axes to move the peak along only one axis at a time, or use the arrow keys to move the peak up, down, left and right.

8. Clear the quick search box above the Compound Table by clicking the red "X" or pressing **Esc**.
9. Click **Show Entire Spectrum**.

Single Peak Compound - Pyruvate

10. Zoom in on the single peak near 2.40 ppm.
11. Right-click on the peak, and click **Search for Compounds Near 2.36 ppm**.
12. In the Compound Table, select pyruvate, since it has the highest Maximum Concentration.
13. Use **Fit Automatically** to suggest a fit for pyruvate (press the space bar).
14. Adjust the pyruvate signature (blue) as needed to match it to the corresponding peak in the spectrum (black).
15. Clear the quick search box.
16. Click **Show Entire Spectrum**.

Water Suppression Effects - Profiling Alanine

17. Zoom in on the doublet near 1.50 ppm.
18. Right-click on the doublet, and click **Search for Compounds Near 1.47 ppm**.
19. In the Compound Table, select alanine.
20. Use **Fit Automatically** to suggest a fit for alanine (press the space bar).
21. Adjust the height of the alanine doublet (blue) as needed to match the corresponding doublet in the spectrum (black).

    You should not normally increase the height of a signature above the black line, so use the right-most peak to determine the appropriate height.
22. Move to the second cluster of alanine (3.8 ppm) using the Cluster Navigator, and adjust its frequency as needed to match the spectrum.

    Since the second cluster is much closer to water than the first one, it is more sensitive to side effects of the water suppression pulse used to acquire the spectrum. Thus, the cluster at 1.5 ppm is a good choice for determining the concentration of alanine, while the cluster at 3.8 ppm must be fit using only frequency adjustments.

    For more details on handling water suppression effects, see "Water Suppression".
23. Clear the quick search box.
24. Click **Show Entire Spectrum**.

Profiling the Remaining Compounds

25. Using the techniques discussed above, profile the remaining nine compounds in the spectrum. You can check your results for all of the compounds against the table at the end of this section. If you are unsure what the finished profile should look like, open the file `Basic End.cnx` to view this spectrum with profiling completed.

    Note that in one instance (3-indoxylsulfate), Profiler is unable to suggest a fit via **Fit Automatically**, due to the atypical shape of the cluster at 7.3 ppm. For this

compound, choose a concentration that provides the best match to the other clusters, then adjust frequencies of each cluster to best match the spectrum.

Some clusters are more reliable than others when determining the concentrations (i.e. heights) of multi-cluster compounds. When you determine concentrations manually, try to use clusters that meet as many of the following criteria as possible:

- Minimal overlap. Ideally, the cluster should have no other clusters overlapping.
- More protons. If a cluster corresponds to three protons (such as a methyl group), the signal for that cluster is stronger than the signal for a cluster corresponding to only one proton.
- Undistorted. If a cluster appears near water (around 4.73 ppm), its signal may be distorted by the presence of the water peak. Further distortions may also be caused by a poor baseline, poor phasing, a high signal-to-noise ratio, solvent suppression, or overly aggressive processing corrections. Use distorted clusters only when no alternative is available, and recognize that concentrations based solely on distorted clusters may be unreliable.

Table 2.1. Expected Concentrations for Basic Start.cnx

Compound	Conc (µM)	Compound	Conc (µM)
3-Indoxylsulfate	434.9	Fumarate	155.6
4-Hydroxy-3-methoxymandelate	49.1	Isopropanol	14.4
Acetate	613.8	Niacinamide	529.2
Alanine	570.9	Pantothenate	94.7
Citrate	432.1	Pyruvate	598.9
DSS (Chemical Shape Indicator)	481.9	Urea	147094.0
Formate	321.2		

2.3 Advanced Profiling Techniques

Real samples are rarely as simple as the previous example, and usually contain compounds whose signatures overlap. Although profiling becomes more challenging in these cases, the strategy remains the same; profile easily identifiable compounds first, then use the contributions of those compounds to refine your efforts at profiling the overlapped regions.

There is a certain artistry to efficiently profiling a complex spectrum. Practice is key; try profiling progressively more complex spectra until you develop a level of proficiency. More detailed discussions of profiling strategies are available in the Chenomx NMR Suite user manual, and in Chapter 3, *Targeted Profiling* .

Getting Started

1. Open **Profiler**.
2. Open the file called `Advanced Start.cnx`.

Although completely non-overlapping compounds are easy to profile, they cannot give any further insight into the shape of other compounds in the spectrum. Profile these compounds first, so that you can focus on the more challenging regions of the spectrum. Since we covered the relevant techniques in "Basic Profiling Techniques" , they will not be covered in detail here.

Profiling Compounds using Basic Profiling Techniques

3. Using the basic techniques discussed in "Basic Profiling Techniques", profile the following twelve compounds.
    - 3-Indoxylsulfate
    - 4-Hydroxy-3-methoxymandelate
    - Acetate
    - Citrate
    - Dimethylamine
    - DSS (Chemical Shape Indicator)
    - Formate
    - Fumarate
    - Niacinamide
    - Pyruvate
    - Threonine
    - Trimethylamine

Once you have profiled the non-overlapped compounds, a more systematic approach will help in profiling the remainder. For this tutorial, start at the right-most edge of the spectrum, and move to the left.

Fitting Compounds with Overlap

4. Zoom in on the two single peaks near 0.90 ppm.

    These peaks correspond to methyl groups in the structure of pantothenate (the highest Maximum Concentration in this region of the spectrum). Methyl groups can be very useful in setting an appropriate concentration for a compound, as they are usually easy to locate.

5. Use **Fit Automatically** (press the space bar), then adjust the concentration of pantothenate to match the spectrum in this region.
6. Click the location of the next pantothenate cluster (2.4 ppm) in the Cluster Navigator.
7. Adjust the frequency of the cluster to match the signature (blue) to the spectrum (black). Although the left-most peak of this cluster overlaps with 2-oxoglutarate, the remaining two peaks provide enough information to establish an appropriate frequency for the cluster.

    It is good practice to completely finish fitting an overlap region before moving on to the next one. Since the only other compound in this region is 2-oxoglutarate, it is the next logical compound to consider.

8. In the Compound Table, select 2-oxoglutarate, and use **Fit Automatically**.
9. Adjust the 2-oxoglutarate cluster near 2.43 ppm as needed to match the spectrum.

    Notice that the sum line (red) at 2.417 ppm now matches the spectrum more closely than it did with only pantothenate adjusted.

    Since 2-oxoglutarate is quite simple, you can finish profiling it before continuing with pantothenate.

10. Move to the remaining 2-oxoglutarate cluster (3.0 ppm) using the Cluster Navigator, and adjust the frequency of the cluster as needed to match the spectrum.
11. Select pantothenate again.

    You can simply click on the sum line (red) to select the nearest compound. Click repeatedly to cycle through the contributing compounds in an overlap region.
12. Move to the next pantothenate cluster (3.4 ppm) using the Cluster Navigator, and adjust the frequency of the cluster at 3.39 ppm to fit the spectrum.
13. In the Compound Table, use **Fit Automatically** on fucose and taurine, and then select taurine.
14. Move to the taurine cluster near 3.3 ppm, and adjust it as needed to match the spectrum.
15. Return to the taurine cluster near 3.4 ppm, and adjust its frequency as needed to match the spectrum.

    In overlapped regions like this, the sum line (red) becomes a better indicator of the quality of your profile in the region, so check the match of the sum line to the spectrum before proceeding.
16. Select fucose, and use the Cluster Navigator to display the clusters near 1.2 ppm.

    Click and drag across the series of clusters in the Cluster Navigator to view the entire group.
17. Adjust the concentration of fucose so that the clusters near 1.2 ppm match the spectrum. The cluster at 1.24 ppm provides the best reference to establish the concentration for fucose.
18. Return to the fucose cluster near 3.4 ppm, and adjust its frequency as needed to match the spectrum, using the sum line to evaluate your progress.
19. Select pantothenate again, and use the Cluster Navigator to view the cluster near 3.5 ppm.
20. Adjust the frequency of the pantothenate cluster at 3.51 ppm as needed to match the spectrum.
21. Move to the pantothenate cluster near 4.0 ppm, and adjust its frequency as needed to match the cluster to the nearest peak in the spectrum.

    The profiled peak for pantothenate in this region does not completely match the area available, due to overlap with serine. Once you have profiled serine, this peak will match better.
22. In the Compound Table, use **Fit Automatically** on glycolate and serine, and then select serine.
23. Use the Cluster Navigator to display the serine cluster near 3.8 ppm, and adjust its concentration to better match the spectrum, using the sum line to evaluate your progress.
24. Use the Cluster Navigator to display the serine clusters at 4.0 and 3.9 ppm. Remember that you can click and drag across several clusters to display all of them.
25. Adjust the frequencies of the remaining two serine clusters.

    Notice that the sum line over the pantothenate cluster in this region now yields a much better match to the spectrum.

26. Select glycolate, and adjust the single glycolate peak as needed to match the spectrum.
27. Select fucose, display the two clusters nearest 3.7 ppm, and adjust their frequencies as needed to match the spectrum.
28. In the Compound Table, select alanine and use **Fit Automatically**.
29. Display the alanine cluster near 1.5 ppm, and adjust it as needed to match the spectrum.

    Use the right-most peak to set the height for the cluster, much like you did for the basic spectrum.
30. Return to the alanine cluster near 3.8 ppm, and adjust its frequency as needed to match the spectrum, using the sum line to evaluate your progress.
31. Adjust the frequencies of each of the fucose clusters near 3.8 ppm. Try finding individual peaks in each cluster that match peaks in the spectrum, and lining them up.
32. Adjust the frequencies of each of the remaining fucose clusters as needed. If there is not enough of a pattern visible in the spectrum to which you can match a cluster, leave the cluster in its original position.
33. In the Compound Table, select urea and and use **Fit Automatically**.
34. Adjust the urea cluster near 5.8 ppm as needed to match the spectrum.
35. Check the overall shape of the spectrum to verify that the sum line lies generally along the spectrum line.

You can check your results for all of the compounds with the following list of concentrations. If you are unsure what the finished profile should look like, open the file `Advanced End.cnx` to view this spectrum with profiling completed.

Table 2.2. Expected Concentrations for Advanced Start.cnx

Compound	Conc (µM)	Compound	Conc (µM)
2-Oxoglutarate	620.9	Fumarate	155.5
3-Indoxylsulfate	448.7	Glycolate	852.1
4-Hydroxy-3-methoxymandelate	50.2	Niacinamide	568.0
Acetate	616.3	Pantothenate	98.7
Alanine	583.7	Pyruvate	588.8
Citrate	452.7	Serine	1185.2
DSS (Chemical Shape Indicator)	481.9	Taurine	1196.3
Dimethylamine	655.7	Threonine	424.1
Formate	328.3	Trimethylamine	618.8
Fucose	618.2	Urea	332288.8

Bonus compounds: There are at least two additional compounds present in trace quantities in this spectrum. Identify and profile these compounds and check their concentrations here:

Suberate (60.6 µM)

Isopropanol (13.8 µM)

Chapter 3. Targeted Profiling

Targeted profiling involves matching a series of compounds to an experimental spectrum. Practically, targeted profiling includes the two tasks of identifying a compound or compounds that you would like to profile, and matching the individual clusters of each compound to their respective regions of the spectrum. Identifying a compound to profile is simply a matter of displaying the compound and visually comparing its signature with visible patterns in the spectrum, remembering that you can adjust both height (concentration) and position (frequency) during the profiling process. There are several methods of filtering and sorting the compound list to include compounds that are most likely to appear in a given region of the spectrum.

Profiling individual clusters in a region of the spectrum requires some care and attention; the most important facet of any profiling technique is applying it consistently to all spectra in a dataset. Profiling can involve a variety of methods, depending on the intensity of the peaks, the presence of other peaks in the region, the degree of overlap with other peaks, and the presence of other clusters from the same compound elsewhere in the spectrum. Some of these methods are described below; you may discover other methods as you analyze more spectra.

3.1 Identifying Compounds

There are a variety of methods that you can use to help you identify compounds that are present in any given spectrum. Some are implemented directly in the Profiler module, while others may involve some literature review or external reference.

Each of the following techniques is essentially a method of limiting the list of potential compounds for a given region of the spectrum. You must make the final decision as to whether or not a particular compound is represented in the spectrum by visually comparing the signature for the compound to the patterns visible in the spectrum. Remember that each cluster can be moved somewhat to either side of its starting position (inside its transform window), and the overall height of the compound can be adjusted.

Frequency Searches

You can use **Search for Compounds** to force the Compound Table to show only compounds that appear at a certain frequency, or within a certain frequency range. To quickly search based on a single frequency, right-click on the spectrum, and click **Search for Compounds Near x.xx ppm**. Similarly, to search based on a frequency range, select a region, then right-click inside the selected region and click **Search for Compounds in this Region**.

A frequency search will display all compounds that have transform windows overlapping the selected frequency or frequency range. Since transform windows are a function of spectrum pH, the number of compounds displayed by a frequency search can vary based on your spectrum pH.

Text Searches

You can search the Compound Table based on simple text, too. Just type your search text into the Quick Search text box at the top right corner of the Compound Table. Searching can help you look for a particular compound, or a group of compounds with similar names, like all compounds containing "acetate".

Compound Lists

A list of compounds known to occur in samples of the type that you are analyzing can be an invaluable tool to guide your profiling efforts. In many cases, the number of compounds that could be contributing to a specific region of the spectrum can be large, so it can be helpful to know some compounds that are more likely to be present. If you do not have such a list, and are expecting to analyze samples of a particular type on a regular basis, you may find it helpful to develop a list of your own based on representative samples.

For the Profiler sample files, the following compound list guided the profiling process:

2-Oxoglutarate	DSS (Chemical Shape Indicator)	Pyruvate
3-Indoxylsulfate	Formate	Serine
4-Hydroxy-3-methoxymandelate	Fucose	Taurine
Acetate	Fumarate	Threonine
Alanine	Glycolate	Trimethylamine
Citrate	Niacinamide	Urea
Dimethylamine	Pantothenate	

If you have a list of compounds common to a sample type, you may want to create a Compound Set in Library Manager and use it to perform initial profiles of the spectra in your dataset. You can also use the **Remember Stars** and **Restore Stars** features to copy a set of common compounds between your spectra.

Test Mixtures

As you profile a series of spectra in a larger dataset, you will notice that fitting some mixtures of compounds is difficult. You may want to try running controlled test mixtures of those compounds to establish difficult or unexpected behaviours under your experimental conditions, and then use those behaviours to guide your profiles of the larger dataset.

3.2 Profiling Clusters

Once you have selected a compound to profile, you need to match the individual clusters of the compound in their respective regions of the spectrum. There are several common approaches to profiling clusters, and in a typical spectrum, more than one method will be necessary. Try to maintain a consistent approach to profiling spectra in a dataset, as this will help you obtain accurate and consistent results from your analysis.

Compound Line

The simplest way to match a cluster to a region of the spectrum is to adjust the cluster so that the compound line (blue by default, unless you have assigned custom colors) matches the spectrum line (black). The compound line is most useful for isolated, low-intensity clusters. If you profile all of the clusters for a compound using only the compound line, the measured concentration of the compound may be unreliable, particularly if other clusters are nearby; the identity can still be considered accurate if the cluster shapes are clearly discernible. Using the compound line on tall, isolated clusters generally provides accurate identity and quantity.

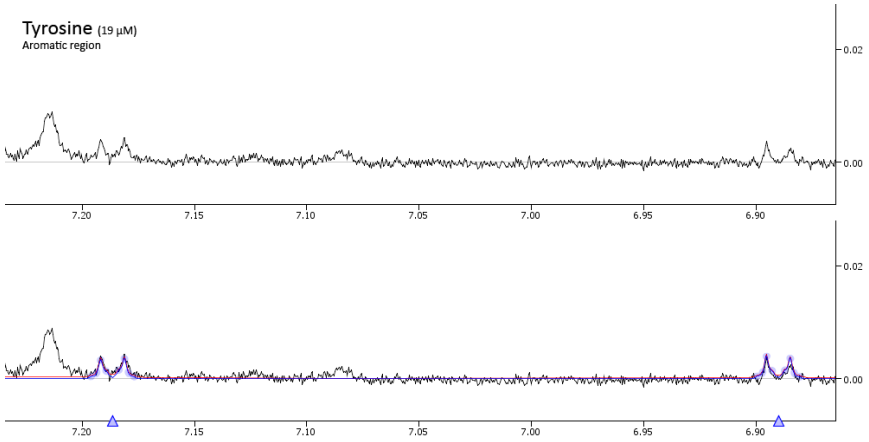


Figure 3.1. Apart from these clusters in the aromatic region, none of the other peaks for tyrosine are clearly visible. Although it is clear that tyrosine is present, the quantity of 19 µM should be considered approximate.

Subtraction Line

For complex regions shapes, use the subtraction line to determine an optimal fit for the cluster, where possible. Adjust the cluster height and position to remove the influence of the adjusted cluster while approximating a normal spectrum with the subtraction line. Using the subtraction line is an advanced technique offering some of the most accurate shapes available, but requires more time than other, simpler techniques.

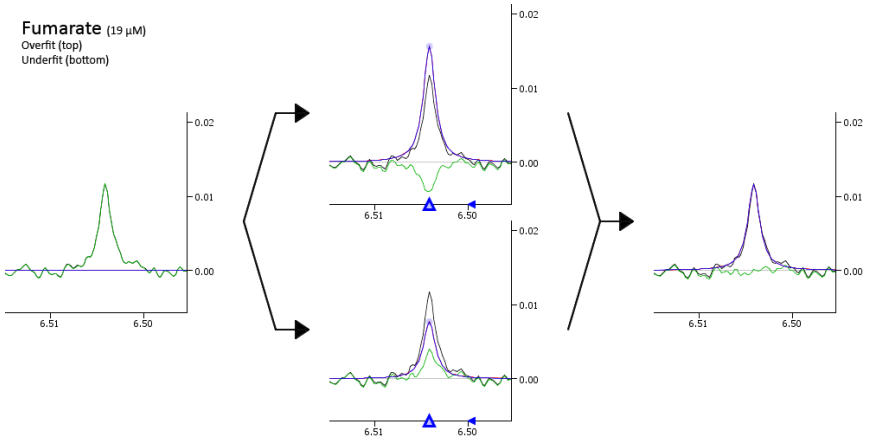


Figure 3.2. The overfit subtraction line (top center) shows a negative residual peak, and results in a concentration of 25 µM. The underfit subtraction line (bottom center) leaves a positive residual peak, and results in a concentration of 12 µM. The accurate profile (far right) results in a subtraction line that approximates a smooth baseline through the area, indicating that the signal has been effectively subtracted from the spectrum.

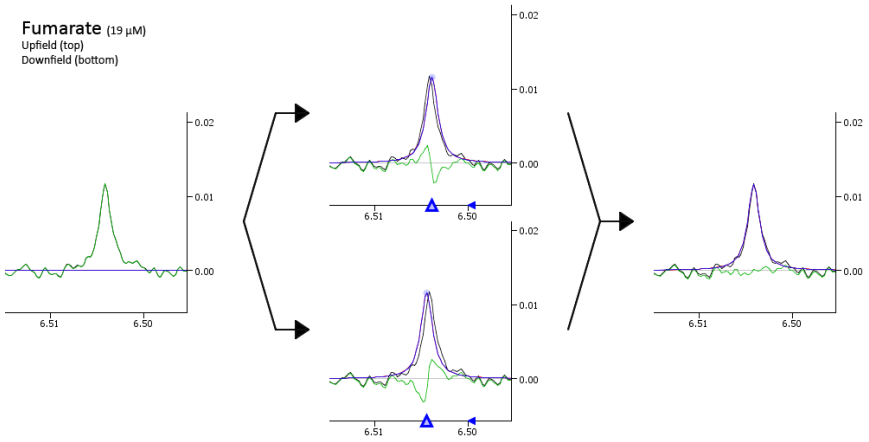


Figure 3.3. Inaccurate positions result in a combination of over- and underfits: A negative residual peak on the right suggests the cluster should be moved left, or downfield (top center). A negative residual peak on the left suggests the cluster should be moved right, or upfield (bottom center). Both identity and quantity can be considered reliable in the accurate profile (far right).

Sum Line

You will encounter situations in which the cluster you need to profile overlaps one or more clusters from other compounds; regions fitting this description are often called "busy regions". You often need to profile compounds that have overlapped clusters in groups, ensuring that the combined shape (sum line) matches the spectrum line.

First, identify likely compounds contributing to the overlapped region. Where available, use non-overlapped peaks, including those in other regions of the spectrum, to establish concentration ranges for the identified compounds. Use partially-exposed peaks to establish positions for each of the overlapped clusters. Starting with the approximate shape thus obtained, fine-tune the position and height of each of the overlapped clusters to optimize the overall shape in the region.

Generally, profile multiplets before singlets in overlap regions, as they are easier to position accurately. If all of the clusters for a compound are in overlap regions, the profile may be unreliable, especially if most or all of the clusters are low-intensity singlets.

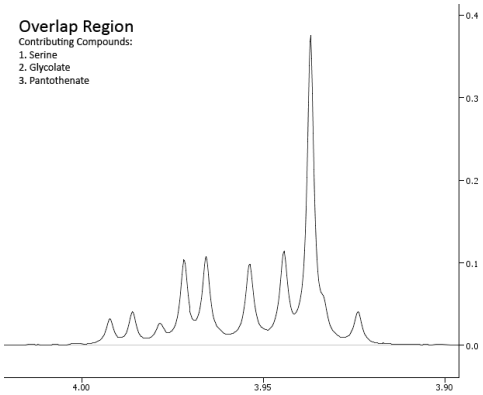


Figure 3.4. Using frequency filters and sorting by maximum concentration lets you identify serine, glycolate and pantothenate as candidate compounds. Visual comparison of the signatures to the spectrum line confirms the identifications.

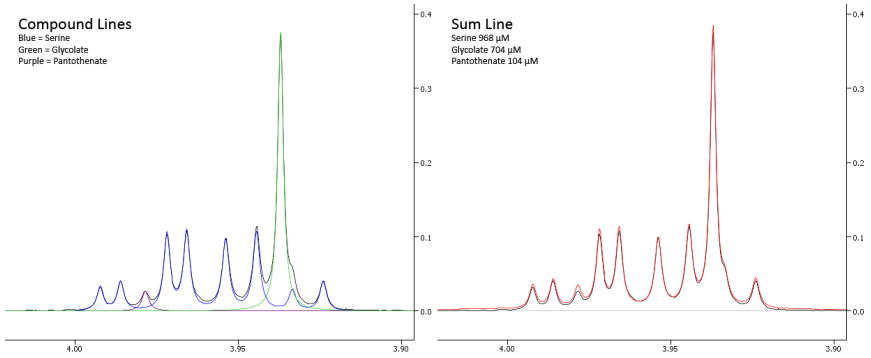


Figure 3.5. Profiling each compound individually using the basic method (simple match to spectrum line) yields reasonable concentrations, but the sum line doesn't quite match.

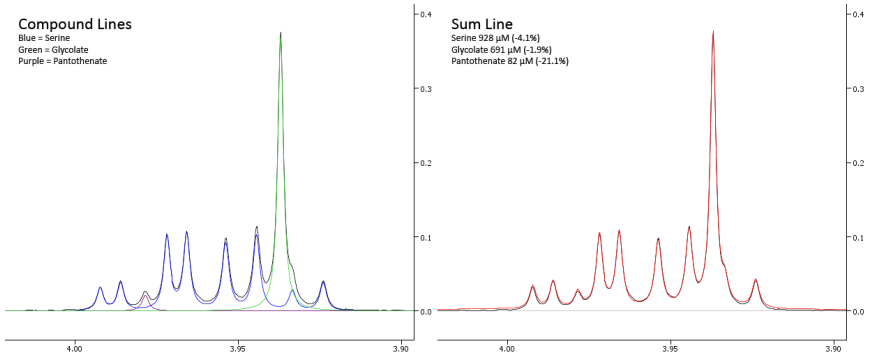


Figure 3.6. Considering all the compounds together, along with confirmation using other clusters of serine and pantothenate, gives a better match. Also, note the more than 20% change in pantothenate concentration!

Water Suppression

Spectra of aqueous samples are often acquired using a water suppressing pulse sequence, since the water signal would otherwise drown out many useful signals. The water suppression pulse can also partially suppress other resonances occurring near the frequency of the water signal. When you profile compounds with multiple clusters, use clusters well away from the water signal to determine compound quantities. This may result in a slight "overfit" (sum line higher than spectrum line) of the clusters closer to water, but gives you more consistent and accurate concentrations.

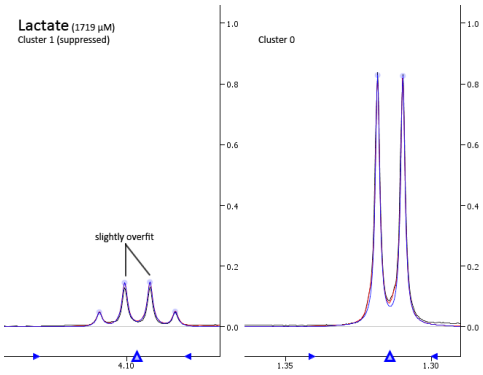


Figure 3.7. Since cluster 0 (right) is quite far from the water peak, it experiences little suppression. Profiling lactate based on cluster 0 results in a slight but acceptable overfit of cluster 1 (left).

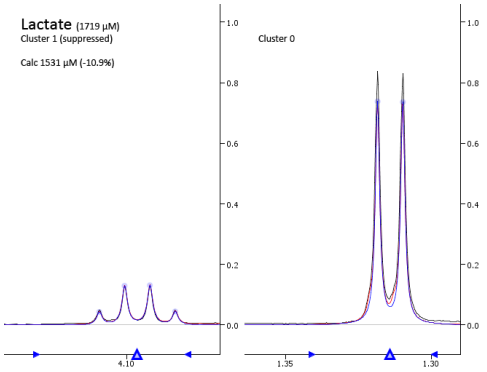


Figure 3.8. Profiling lactate based on cluster 1 (left) incorrectly reduces the reported concentration by almost 11%.

Best Guess

When you profile a compound with multiple clusters, you may encounter a cluster that is difficult to profile accurately, due to a lack of prominent peaks in the signature or in the spectrum, or both. In this case, use minor adjustments to approximately match the sum line to the spectrum line; the goal here is mostly aesthetic, since very little information is available to produce an accurate profile. If all of the clusters for a compound must be profiled using this technique, the profiled compound (both identity and quantity) should be considered unreliable.

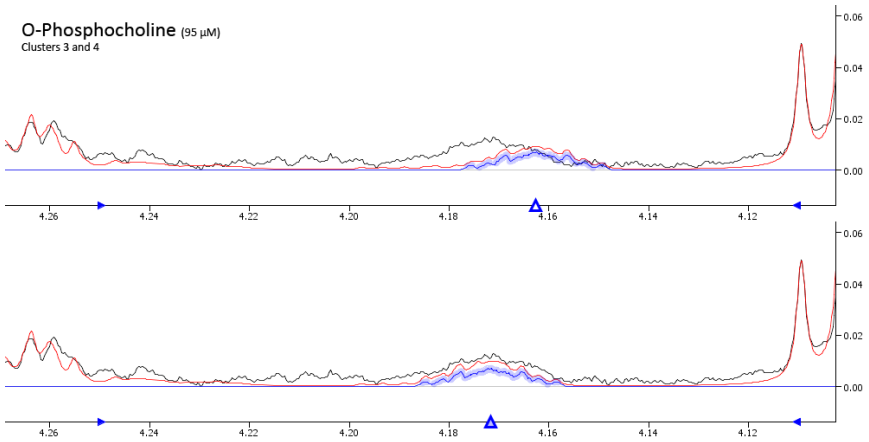


Figure 3.9. The large cluster near 3.2 ppm was sufficient to establish the identity and quantity of O-phosphocholine. Although there are no obvious peaks that can be used to align clusters 3 and 4 (top), it seems reasonable to move them over to fit more neatly under the nearby "hump" in the spectrum (bottom).

No Fit

When profiling some compounds, you may find that a cluster is not visible in the signature or the spectrum, or both, at concentrations consistent with the spectrum. Since no information is available to help you profile the cluster, the best course is to leave the cluster at its starting position. If all of the clusters for a compound fall into this category, the compound should be considered not detected in the sample.

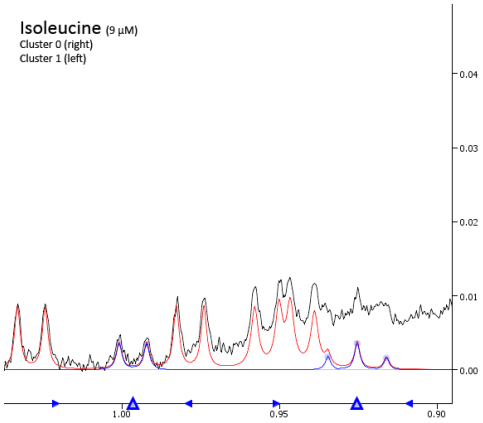


Figure 3.10. The doublet near 1 ppm provides enough information to set a concentration for isoleucine, and the triplet near 0.92 ppm lets you confirm the identification. It is reasonable that isoleucine is present, at or near 9 µM.

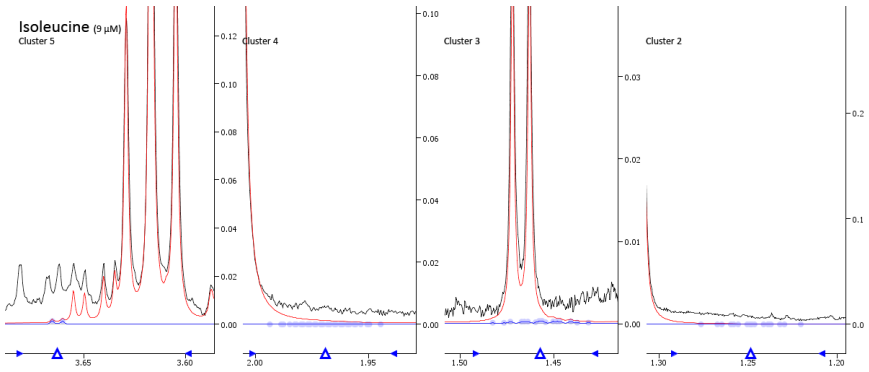


Figure 3.11. There is no clear information elsewhere in the spectrum to guide the placement of the remaining clusters, so they are left at their default positions. The initial assessment of isoleucine at 9 µM is still consistent with the spectrum.

Chapter 4. Batch Fit

4.1 Introduction

The batch fit feature is a profiling tool that can be used to speed up your profiling if you have several spectra from similar matrices within a study. You can select multiple spectra to be automatically profiled with a set of compounds that you choose. This tool uses an algorithm similar to the **Fit Automatically** (space bar) function, but performs the automatic fit with multiple compounds in multiple spectra. The process of automatically fitting multiple compounds in multiple spectra works best when you first spend time manually identifying the compounds present in one of the spectra in your study. Here in this tutorial chapter, we will outline and guide you through an example of how to use this feature.

4.2 Batch Fitting Multiple Spectra

Before beginning this process, make sure all of your spectra are processed and calibrated with the correct CSI using Processor. In this tutorial this is done for you and the processed spectra can be found in /Samples Files/Batch Fit folder.

1. Open **Profiler**.
2. Open the file called `Batch Reference.cnx`. This spectrum has already been profiled. The compounds identified and their chemical shifts in this spectrum will be used to aid **Batch Fit** in profiling your unprofiled spectra.
3. Select **Tools > Batch Fit**.
    a. Step 1: Select files to fit.

       Select individual files or a folder of files to fit automatically. For the tutorial, select the files `Sample 1.cnx-Sample 5.cnx`

    b. Step 2: Select the compounds to fit automatically.

       Three methods can be used to **Batch Fit** the previously selected files.

        i. Choose Compounds from a Profiled Spectrum: This is the recommended method for batch fitting. This option uses a previously profiled spectra as a reference for profiling the remaining spectra. The "Use transforms from this spectrum as a starting point" checkbox takes into account any clusters that you have transformed manually in the profiled spectrum, and starts the auto fit in these transformed regions. If not selected profiler will **Fit Automatically** with the modeled cluster positions from the pH Sensitive Chenomx Reference Compounds. For this tutorial we will be using this method of selecting compounds. Use the file field to select the `Batch Fit Reference.cnx` file and ensure that the checkbox is selected.

        ii. Choose Chenomx Reference Compounds: Select a Chenomx library at a given frequency and choose the compounds you wish to fit.

        iii. Choose Compounds From: Select a previously created compound pack to fit.
4. Step 3: Refine your previous compound selection.

This list will show all the compounds that profiler will be batch fitting. Compounds can be removed from this list and placed in the “Will Not Be Changed” list to the left by selecting them and clicking the green arrow that points to the left. Moving compounds to the “Will Not Be Changed” list prevents changes being made by the auto fit to compounds that you have already previously fit in the spectra. For this tutorial, leave all of the compounds in the "Will Be Fit Automatically" list

5. Step 4: Confirmation

   This is a summary page to confirm the choices you are making. If you want to go back and review, now is the time to do so. Once you click **Finish**, permanent changes will be applied and saved to the files you have chosen to batch fit. If you want to allow yourself a chance to undo the changes you must make a copy of your spectra files before clicking **Finish** on this page.

4.3 Reviewing Spectra

Once **Batch Fit** is complete, we now want to review each spectra. Profiler will suggest concentrations and peak positions that are acceptable however; it is important to review the spectra. We suggest you go through each file to review the fits of each metabolite, and fit any metabolites that could not be fit automatically. An easier way to do this is to use the Files panel.

1. To ensure this feature is visible, go to **View > Show left Sidebar**.
2. Change the left sidebar to show the **Files** panel by changing the pull down menu on top of the sidebar.

Review the fit for each metabolite. Double clicking on another file in the **Files** panel will open that file in your current Profiler window. You will notice the selection of the metabolite you are reviewing and the zoom location on the graph is preserved as you move from file to file. The software will also prompt you to save your work, if you make any adjustments to a file you are reviewing.

Batch Fit may not work for compounds at low concentrations, and as such you may have to manually fit these compounds that cannot be automatically fit.

Chapter 5. Spin Simulator

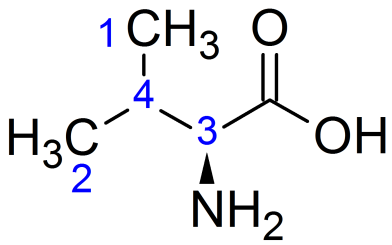
5.1 Introduction

You can use Spin Simulator to create spin simulations of compounds that you would like to add to your library. Simulations that you create in Spin Simulator can be imported into Compound Builder to create compound signatures that you can then add to your library.

You may find it is helpful to have the molecular structure of the compound you are simulating available for reference purposes.

5.2 Simple Simulation - Valine

To become familiar with the various tools available in Spin Simulator, as well as with the basic process involved in creating a simulation, you will simulate a simple compound, valine.



Aside from exchangeable protons, like the carboxylic acid and amine protons, there are four types of protons expected to have visible signals for this compound, marked as 1 through 4 in the structure above. The two methyl groups (1 and 2) and one of the methine groups (3) will each couple to the remaining methine group (4). Thus, in the spectrum for valine, you would expect to see three doublets, each coupled to one complex cluster (rigorously, a quartet of quartets of doublets, or qqd).

Simulating Valine

1. Assign protons in the structure of the compound to signals in the spectrum. For valine, the assignments are as follows:
    a. Methyl signals (1 and 2) appear at 0.978 and 1.031, each coupled to one of the the methine signals (4).
    b. Methine signal (3) appears at 3.604 ppm, and is coupled to the other methine (4).
    c. Methine signal (4) appears at 2.264 ppm, and is coupled to each of the other three signals.
2. Open **Spin Simulator**.
3. Overlay the valine spectrum, called `Valine.cnx` and make sure that the **Spin Definition** pane is visible in a sidebar.

    Note: the files mentioned in this chapter are part of the sample file set that came packaged with this document. They can be found in the `Sample Files\Spin Simulator` folder.

4. Add spin definitions for each of the signals from the assignment above, starting from the right-hand side of the spectrum (low numbers) and moving left (to higher numbers).

   You now have Spin Definition 0 and Spin Definition 1 for the methyl groups (3 protons each, at 0.978 and 1.031 ppm), and Spin Definition 2 and Spin Definition 3 for the methine groups (1 proton each, at 2.264 and 3.604 ppm).

5. Zoom in to the two methyl signals near 1.0 ppm.

   You can click and drag across the two corresponding entries in the Spin Navigator to provide a useful zoom level.

6. Measure the distance between the pairs of peaks in each doublet using the Select Region tool.

   The right-hand doublet measures about 7.02 Hz, while the left-hand one measures about 7.07 Hz.

7. Select Spin Definition 0 (0.978 ppm) by clicking on it in the graph, or selecting it in the Spin Definitions list

8. Right-click on Spin Definition 0 on the graph or in the list and click **Add New J-Modifier...**

9. Enter a J-value of 7.02 Hz, select Uncoupled Split with a split count of 1, and click OK.

10. Adjust first spin definition (blue) to fit the spectrum (black). You can adjust freely by clicking and dragging the peak shape, or use either the controls on the horizontal and vertical axis or the up, down, left and right arrow keys to restrict your adjustment to specific dimensions and directions.

11. Adjust the width of the doublet using the controls to either side of the spin definition, so that it better matches the spectrum.

    A width of about 1.2 to 1.3 Hz is sufficient.

12. Select Spin Definition 1 (1.031 ppm).

13. Add a J-modifier to Spin Definition 1.

    Use a J-value of 7.07 Hz, and select Uncoupled Split with a split count of 1.

14. Adjust Spin Definition 1 to fit the spectrum.

    Once again, a width of 1.2 to 1.3 Hz is sufficient.

15. Zoom to Spin Definition 3 (3.604 ppm) using the Spin Navigator or the Spin Definition pane.

16. Measure the distance between peaks in the doublet.

    This doublet measures about 4.36 Hz.

17. Add a J-modifier to Spin Definition 3.

    Use a J-value of 4.36 Hz and select Uncoupled Split with a split count of 1.

18. Adjust Spin Definition 3 to fit the spectrum.

    This doublet has a width of around 1.1 Hz.

19. Zoom to Spin Definition 2 (2.264 ppm) using the Spin Navigator or the Spin Definition pane.

The resulting view does not reveal much of the actual spectrum, so adjust it to get a better view.

20. Click **View > Zoom > Set Zoom**, and enter 2.23 to 2.30 ppm for the X Axis, and -0.05 to 0.87 si for the Y Axis.
21. Add a J-modifier to Spin Definition 2. Select Coupled, to Spin Definition 0, using the uncoupled split of 7.02 Hz.

    The spin definition is now linked to one of the methyl groups; notice that it has split to a quartet (n=3).
22. Add two more J-modifiers, one coupled to Spin Definition 1 using the uncoupled split of 7.07 Hz, and the other coupled to Spin Definition 3 using the uncoupled split of 4.36 Hz.

    Notice that with each added J-modifier, the spin definition becomes more complex, and matches more closely with the spectrum.
23. Adjust Spin Definition 2 to better fit the spectrum.

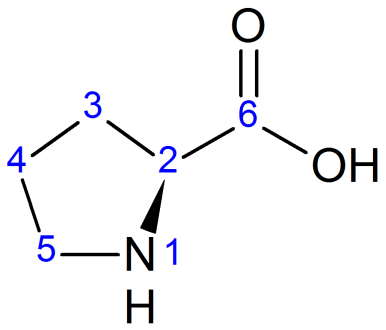
    This spin definition has a width of about 1.5 Hz.
24. Notice that changing the height of either spin definition changes the height of the whole simulation; you cannot obtain a perfect height match with all four spin definitions at the same time. The discrepancy is normal, and can be corrected in Compound Builder prior to adding the compound to your library (see "Simple Compound - Valine" for details).

    For now, simply choose one of the spin definitions with which to fit the simulation height.
25. Save the simulation file.

    If you would like to review the sample simulation of valine, do not replace the file called `Valine.xss`.

5.3 Complex Simulation - Proline

Now that you have developed a basic familiarity with some of the tools available in Spin Simulator, you are ready to use them to simulate a more complex compound, proline.



The spectrum of proline is much more complex than the spectrum of valine. The protons at each position couple to all of the protons at adjacent positions. Also, the protons at each position are diastereotopic, meaning that they can couple with each other (geminal coupling), as well as each coupling to adjacent protons. While this means that splitting patterns will be based exclusively on doublets (n=1), it also means that you need to be aware of a lot more coupling interactions.

Simulating Proline

1. Assign protons in the structure of the compound to signals in the spectrum. Note that this is often one of the largest tasks in simulating a new compound. For proline, the assignments are as follows (protons labelled 'a' project above the plane of the page, while those labelled 'b' project below the plane):
    a. The signal for proton 4b appears at 1.979 ppm, and couples to protons 3a, 3b, 4a, 5a and 5b (Spin Definition 0, or SD0).
    b. The signal for proton 4a appears at 2.012 ppm, and couples to protons 3a, 3b, 4b, 5a and 5b (Spin Definition 1, or SD1).
    c. The signal for proton 3b appears at 2.058 ppm, and couples to protons 2, 3a, 4a and 4b (Spin Definition 2, or SD2).
    d. The signal for proton 3a appears at 2.339 ppm, and couples to protons 2, 3b, 4a and 4b (Spin Definition 3, or SD3).
    e. The signal for proton 5a appears at 3.328 ppm, and couples to protons 4a, 4b and 5b (Spin Definition 4, or SD4).
    f. The signal for proton 5b appears at 3.413 ppm, and couples to protons 4a, 4b and 5a (Spin Definition 5, or SD5).
    g. The signal for proton 2 appears at 4.121 ppm, and couples to protons 3a and 3b (Spin Definition 6, or SD6).
2. Open **Spin Simulator**.
3. Overlay the proline spectrum, called `Proline.cnx`.
4. Add spin definitions for each of the protons listed in the assignment above, making sure that the number of protons for each is set to one. Although you do not need to add the spin definitions in the indicated order to complete the tutorial, doing so will make the tutorial easier to understand, since the Spin Definitions are labelled based on the order they are added.
5. Zoom in to Spin Definition 6 (4.121 ppm).
6. Measure the two coupling constants represented in this region of the spectrum, using the spectrum line (black) as your reference. Specifically, find the distances from an outermost peak to the adjacent peak and from an outermost peak to the second peak in. The distances in this spectrum are 8.79 Hz and 6.52 Hz.
7. Add one J-modifier to Spin Definition 6 for each of the measured distances. Use uncoupled splits, with split counts of 1 and J-values of 8.79 and 6.52 Hz.

You know that each of these couplings will link to single protons, so you can use a split count of 1 for each of them.

8. Adjust the frequency, height and width of Spin Definition 6 to better fit the spectrum.
9. If you need to adjust the J-values for Spin Definition 6, select the J-modifier in the Spin Definition view, and use the J-Value Editor appearing under the Spin Navigator to fine-tune the J-value.
10. Zoom in to Spin Definition 4 and Spin Definition 5.

Based on the assignment above, there are three coupling constants associated with each of these spin definitions. One can be easily measured as the distance between an outermost peak and the second peak in; this measurement yields around 11.58 Hz for both spin

definitions, suggesting that this is the coupling constant linking these two spin definitions. In addition, the value itself (11.58 Hz) is consistent with the geminal coupling relationship between the protons responsible for these signals.

11. Add a J-modifier to Spin Definition 5, and couple it to Spin Definition 4 using a New Split, with a J-value of 11.58 Hz.

The remaining two coupling constants for Spin Definition 5 are both near 7 Hz (the distance from an outermost peak to the adjacent one), but it is not clear what the precise values should be. You can set them to 7 Hz to approximate the correct value, and use the J-Value Editors to refine the values visually.

12. Add two J-modifiers to Spin Definition 5 as uncoupled splits, each with a J-value of 7 Hz, and a split count of 1.
13. Use the J-Value Editor to fine-tune the J-modifiers that you just added. Aim for values that center each of the peaks in the spin definition (blue) on the corresponding peaks in the spectrum (black). The resulting values are 7.40 Hz and 6.67 Hz.
14. Adjust the frequency, height and width of Spin Definition 5 to better fit the spectrum.

You can determine the remaining two coupling constants for Spin Definition 4 using similar techniques. The approximate distances are 7.2 Hz.

15. Add two J-modifiers to Spin Definition 4 as uncoupled splits, with J-values of 7.2 Hz each, and split counts of 1.
16. Use the J-Value Editor to fine-tune the J-modifiers. The resulting values are 7.44 and 6.88 Hz.
17. Adjust the frequency, height and width of Spin Definition 4 to better fit the spectrum.

The remaining spin definitions are quite complicated. There is a lot of coupling among them, including some to spin definitions that you have already fit. Some of these links to solved spin definitions can help reduce the complexity of modeling the spin definitions that remain. Start with Spin Definition 3, since it is the last one that is well-isolated from other signals.

Due to the complexity of this signal, it is not immediately clear which measurements you can use to extract coupling constants. However, one clue exists in the geminal relationship between the protons responsible for this signal and Spin Definition 2. You observed a coupling constant of 11.58 Hz previously between geminal protons, so start by looking for a measurement of similar magnitude. Measuring from an outermost peak to a peak near 11.58 Hz gives about 8.7 or 13.7 Hz. The former value does not appear quite large enough for a geminal coupling, so use the latter to couple Spin Definition 3 to Spin Definition 2.

18. Add a J-modifier to Spin Definition 3, and couple it to Spin Definition 2 using a New Split with a J-value of 13.7 Hz.

Spin Definition 3 should also be coupled to Spin Definition 6, based on the original assignments. There are two J-modifiers available to create this link, with J-values of 8.79 and 6.52 Hz. However, based on the structure, you can estimate the dihedral angle between these two protons as larger than that between Spin Definition 2 and Spin Definition 6. A larger dihedral angle implies a larger coupling constant, according to the vicinal Karplus correlation, so use the larger of the two here.

19. Add a J-modifier to Spin Definition 3, and couple it to Spin Definition 6 using the larger of the two available uncoupled splits (8.79 Hz).

Finally, you know that this signal should be coupled to Spin Definition 0 and Spin Definition 1, but the magnitude of the coupling constants is not clear. You do have information from some of the other couplings in the molecule that suggest the coupling constants should be near 7 Hz, so use this as a starting point, and fine-tune using the J-Value Editor. Similarly, based on the dihedral angles among the protons, you can speculate that the coupling constant to Spin Definition 1 will be larger than the coupling to Spin Definition 0, and bias your adjustments accordingly.

20. Add two J-modifiers to Spin Definition 3, one coupled to Spin Definition 0, and the other to Spin Definition 1, each with a J-value of 7 Hz.
21. Use the J-Value Editor to fine-tune the two new J-modifiers. Ensure that the J-value for the coupling to Spin Definition 1 is larger than the J-value for the coupling to Spin Definition 0.

Unfortunately, there is no entirely satisfactory combination of these two coupling constants that results in a good fit of the cluster. However, the coupling constant to Spin Definition 2 is still speculative, so try adjusting that, as well.

22. Continue adjusting the J-modifiers, this time including the coupling to Spin Definition 2 in your adjustments. As before, aim for values that center each of the peaks in the spin definition (blue) on the corresponding peaks in the spectrum (black). The resulting values are 12.99 Hz (to SD2), 7.38 Hz (to SD1) and 6.65 Hz (to SD0).
23. Adjust the frequency, height and width of Spin Definition 3 to better fit the spectrum.

Spin Definition 2 has a coupling pattern similar to Spin Definition 3. You have already coupled it to Spin Definition 3, and there is only one remaining J-modifier on Spin Definition 6 to which you can couple. Also, the coupling constants to Spin Definition 0 and Spin Definition 1 should be of similar magnitude to those you just established for Spin Definition 3, so again, start them at 7 Hz. Note that the Karplus correlation suggests that this time, the coupling constant to Spin Definition 0 should be larger than the coupling to Spin Definition 1.

24. Add a J-modifier to Spin Definition 2, and couple it to Spin Definition 6 using the only remaining uncoupled split (6.52 Hz).
25. Add two J-modifiers to Spin Definition 2, one coupled to Spin Definition 0, and the other to Spin Definition 1, each with a J-value of 7 Hz.
26. Use the J-Value Editor to fine-tune the J-modifiers. Ensure that the J-value for the coupling to Spin Definition 0 is larger than the J-value for the coupling to Spin Definition 1. The resulting values are 7.57 Hz (to SD0) and 6.87 Hz (to SD1).
27. Adjust the frequency, height and width of Spin Definition 2 to better fit the spectrum. Since the spin definition overlaps with others on the right-hand side, use the peaks on the left-hand side to guide your adjustments.

Most of the coupling constants relevant to the last two spin definitions (SD0 and SD1) have already been defined. There is yet another geminal relationship between the associated protons, so couple them using a J-value comparable to the other geminal couplings you have seen (about 13 Hz).

28. Add a J-modifier to Spin Definition 1, and couple it to Spin Definition 0 using a J-value of 13 Hz.

Since the signals for Spin Definition 0 and Spin Definition 1 in the spectrum both appear simpler than you might expect given that they are coupled to five other signals, it seems

reasonable that the remaining two coupling constants for each spin definition will be similar. The uncoupled splits that are available are associated with Spin Definition 4 (7.44 Hz and 6.88 Hz) and Spin Definition 5 (7.40 Hz and 6.67 Hz). Thus, the two larger coupling constants must be associated with Spin Definition 0 and the two smaller with Spin Definition 1, or vice versa. A quick test of each alternative indicates that the former option yields a better approximation of the spectrum.

29. Add a J-modifier to Spin Definition 1, and couple it to Spin Definition 4 using the smaller of the available uncoupled splits (6.88 Hz).
30. Add a J-modifier to Spin Definition 1, and couple it to Spin Definition 5 using the smaller of the available uncoupled splits (6.67 Hz).
31. Add a J-modifier to Spin Definition 0, and couple it to Spin Definition 4 using the remaining uncoupled split (7.44 Hz).
32. Add a J-modifier to Spin Definition 0, and couple it to Spin Definition 5 using the remaining uncoupled split (7.40 Hz).
33. Use the J-Value Editor to fine-tune the J-modifier coupling Spin Definition 0 to Spin Definition 1. The resulting value is 13.23 Hz.
34. Adjust the frequency, height and width of Spin Definition 1 and Spin Definition 0 to better fit the spectrum.
35. Save the simulation file. If you would like to review the sample simulation of proline, do not replace the file called `Proline.xss`.

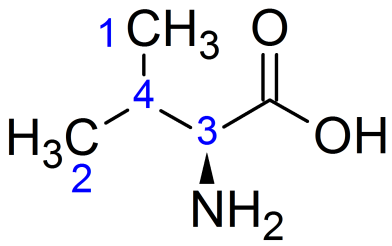
Chapter 6. Compound Builder

6.1 Introduction

You can use the Compound Builder module to build compound signatures that you can add to your library. Although you can build compound signatures from scratch directly in Compound Builder, you can build complex compounds more accurately if you simulate them in Spin Simulator first. The simulation gives you a good starting point from which to build a high-quality compound signature.

6.2 Simple Compound - Valine

To become familiar with the various tools available in Compound Builder, you will build a compound signature for a simple compound, valine. You can use the simulation that you created during the Spin Simulator tutorial, or just use the Spin Simulator sample files provided. If you have not yet completed the Spin Simulator tutorial, you may find it helpful to do so before proceeding with Compound Builder.



The simulation of valine is a model representation of the expected spectrum for valine. Compound Builder helps you to turn this model spectrum into a more accurate representation of an experimental spectrum. For this purpose, the four main areas you need to consider are refining the model, simplifying the model and defining transform windows (and the associated valid pH range). For the best results when you add the compound to your library, you also need to add some descriptive metadata about the compound.

Creating a Compound Signature for Valine

1. Open **Compound Builder**.
2. Import the valine simulation.

   If you have already completed the Spin Simulator tutorial, import the simulation that you created. Otherwise, import the sample simulation, called `Spin Simulator\Valine.xss`.
3. When prompted to calibrate the simulation environment, select Spectrum (`.cnx`), and click the Browse button (...). Select the valine spectrum, called `Valine.cnx`.

   Note: the files mentioned in this chapter are part of the sample file set that came packaged with this document. They can be found in the `Sample Files\Compound Builder` folder.

The compound signature that you build will become part of your compound library. To ensure that the new compound is properly represented in your library, you need to add

some basic information about the compound itself. Some information has been filled in automatically based on the calibration spectrum that you selected.

4. Open the Compound Details dialog.
5. In the General tab, enter your name as the Compound Author, and enter a Sample Concentration of 10 mM.

   For better integration with compounds based on data from multiple spectrometers, including the Chenomx library, you may want to replace the specific magnet frequency obtained directly from the spectrum (799.81 MHz) with the more general 800 MHz.
6. In the Advanced tab, enter the Chenomx Compound ID for valine, 215. This field can be left blank in situations where you don't know the Chenomx Compound ID for your new compound.
7. Enter a valid pH of 6.92. Also, enter 'noesy' as the pulse sequence.
8. Click **OK**.

You will need to set appropriate transform windows before adding the signature to your library. Also, turning on the subtraction line can give you valuable insight into how well the compound fits the experimental spectrum.

9. Click **Compound > Transform Window > Set Default on All Clusters** to set a default transform window (0.006 ppm wide, centered on the cluster center) for every cluster in the compound.
10. Switch to the Legend sidebar, and turn on the subtraction line (green).

Several of the clusters in the imported simulation are quite simple. Focus on matching the simple clusters to the spectrum first, and then deal with the more complex cluster.

11. Move to the cluster near 3.6 ppm using the Cluster Navigator and select it.
12. Adjust the cluster (blue) to fit the spectrum (black). To further refine the shape, adjust the cluster so that the subtraction line is as flat as possible.

When you are adjusting simple clusters, you can get a very good approximate shape for the cluster automatically, using optimization.

13. Move to the clusters near 1.0 ppm using the Cluster Navigator.
14. Select the cluster near 0.98 ppm and click Optimize Selected Peak Shapes.
15. Review the optimized shape, and refine it as needed.

To obtain accurate quantification when you use this compound to analyze spectra in Profiler, it is helpful to recognize that peak heights have a larger effect on the quantification results than peak width or peak area. Thus, when you refine the shape of a cluster, it is important to ensure that the heights of the peaks match the experimental spectrum particularly well.

16. Select the cluster near 1.03 ppm, optimize it, and refine the shape as needed.
17. Move to the cluster near 2.3 ppm using the Cluster Navigator, select it, and optimize the shape.

In this case, optimization cannot provide a better solution than the one you started with. However, when you review the list of peaks in the cluster (click on each to view its contribution to the cluster), you may notice that there appears to be more peaks present than are strictly necessary. Simulation can produce peaks that do not contribute much to

the compound at the current frequency. Part of building a good compound signature is recognizing these extraneous peaks and removing them.

18. Select the first peak in the peak list for the cluster. Using the up and down arrow keys, flip through the list and observe the contributions of each peak.

The first two peaks (2.234 and 2.240 ppm) appear normal, but the next two peaks (both very near 2.243 ppm) appear almost identical, but offset slightly. Near-identical pairs of peaks are excellent candidates for pruning, as the two peaks could be replaced with one without any significant change in the overall shape.

19. Find all of the near-identical pairs of peaks in the cluster, and remove one peak from each pair (6 peaks removed).

Further review reveals that some of the taller peaks in the spectrum are represented by groups of three peaks; one tall central peak flanked by two much shorter peaks. The flanking peaks can also be removed without reducing the shape quality.

20. Find all of the groups of three peaks as described above, and remove the two flanking peaks from each group (6 groups, 12 peaks removed).
21. Optimize the shape of the cluster again.

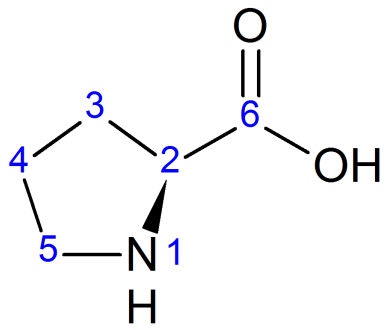
The results of the second optimization are much better, and require only minimal refinement. In addition, you have now reduced the complexity of the model (from 38 peaks to 20), while improving the overall quality of the fit.

Now that the compound matches the spectrum well, you can add proper transform windows to each cluster, build additional signatures for a pH sensitive compound, or make this signature pH sensitive using other signatures as data points.

22. Save the compound file. If you would like to review the sample signature for valine, do not replace the file called `Valine.xcpd`.

6.3 Complex Compound - Proline

Now that you have developed a basic familiarity with the tools available in Compound Builder, you are ready to use them to build a compound signature for a more complex compound, proline.



Creating a Compound Signature for Proline

1. Open **Compound Builder**.
2. Import the proline simulation.

If you have already completed the Spin Simulator tutorial, import the simulation that you created. Otherwise, import the sample simulation, called `Spin Simulator\Proline.xss`.

3. Select Spectrum (`.cnx`), and click the Browse button (...). Select the proline spectrum, called `Proline.cnx`.
4. Open the Compound Details dialog.
5. In the General tab, enter your name as the Compound Author, and enter a Sample Concentration of 10 mM.

   For better integration with compounds based on data from multiple spectrometers, including the Chenomx library, you may want to replace the specific magnet frequency obtained directly from the spectrum (799.81 MHz) with the more general 800 MHz.
6. In the Advanced tab, enter the Chenomx Compound ID for proline, 230. Also, enter a valid pH of 6.93. Enter 'noesy' as the pulse sequence.
7. Click OK.
8. Set default transform windows on all clusters, and make sure that the subtraction line (green) is visible.

Several clusters are quite easy to adjust, and do not overlap with any other clusters, so refine these ones first.

9. Optimize each of the clusters near 4.1, 3.4, 3.3 and 2.3 ppm, respectively, and refine them as needed.

The cluster near 2.1 ppm overlaps slightly with the cluster near 2.0 ppm, but the overlap should not cause any particular difficulty. However, the cluster does include some extraneous peaks, so remove them before proceeding.

10. Move to the cluster near 2.1 ppm, select it, and optimize the shape.
11. Review the contributions of each of the peaks to the overall cluster shape. Delete peaks that appear particularly small relative to the peaks immediately adjacent (6 peaks removed).
12. Optimize the cluster, and refine it as needed.

The remaining two clusters are heavily overlapped, and optimizing them at this point yields unsatisfactory results. You will need to simplify both of these clusters before refining their shapes.

13. Review the contributions of each peak in the cluster at 1.979 ppm to the overall shape of the cluster.
    a. For pairs of near-identical peaks, remove one peak from each pair (8 peaks removed).
    b. For groups containing peaks much smaller than the immediately adjacent peaks, remove the small peaks (13 more peaks removed).
    c. To evaluate the effectiveness of removing a particular peak from the cluster, remove it, and then select all the peaks contributing to the apparent peak in the spectrum. Optimize the subset of peaks, and review the results. If all of the remaining peaks contribute significantly to the optimized shape, then move

on. Otherwise, undo the optimization, and remove another peak from the subset.

14. Review the contributions of each peak in the cluster at 2.011 ppm in the same way as described for the previous cluster (22 peaks removed).
15. Select both clusters, and optimize them together.
16. Refine the shapes of both clusters as needed.

Now that the compound matches the spectrum well, you can add proper transform windows to each cluster, build additional signatures for a pH sensitive compound, or make this signature pH sensitive using other signatures as data points.

17. Save the compound file. If you would like to review the sample signature for proline, do not replace the file called `Proline.xcpd`.

Chapter 7. Building pH Sensitive Compounds

7.1 Introduction

You can use Compound Builder to build pH sensitive compounds similar to the Chenomx Reference Compounds. pH sensitive compounds use expressions incorporating the spectrum pH in place of constant values for certain cluster properties, including cluster centers and transform windows. These expressions allow compounds to partially adapt to the spectrum before you profile it, reducing the time and effort needed to accurately identify compounds in the spectrum.

Before you can create a pH sensitive compound for your Library, you will need some data to work with.

Preparing to create a pH sensitive compound

1. Prepare one signature file to be made pH sensitive (the 'master' signature). You can use the valine signature file that you created in the Compound Builder tutorial (see "Simple Compound - Valine") as a starting point.
2. Prepare a number of other signature files ('partial' signatures) spanning the pH range over which you would like your pH sensitive compound to be valid. For each of these:
   - Set the valid pH to the pH of the original sample (i.e. a different pH value for each 'partial' signature.)
   - Make sure each partial signature has the same number of clusters, in the same order, as the master signature. Rearrange the cluster IDs if necessary (see the Chenomx NMR Suite user manual).
   - Make sure that the cluster centers in each partial signature are as accurate as possible, as these will be used along with the pH values to generate the necessary expressions.

7.2 Building a pH Sensitive Compound

Once you have built the necessary signatures, turning them into a pH sensitive compound signature is quite simple. Remember that the quality of the pH sensitive signature that you build is directly related to the quality of the source signatures, and in particular the master signature.

Creating a pH sensitive version of valine

1. Open **Compound Builder**.
2. Open the valine signature.

   If you have already completed the Compound Builder tutorial, open the signature that you built. Otherwise, open the sample signature, called `Valine.xcpd`.

   Note: the files mentioned in this chapter are part of the sample file set that came packaged with this document. They can be found in the `Sample Files\Compound Builder` folder.
3. Overlay the valine spectrum, called `Valine.cnx`.

4. Open the pH Sensitivities dialog.
5. Click **Load Data Points...**.
6. Select the folder called `pH Data Points` and click **Set Data Points**.
7. Use the tabs at the top of the window to review each cluster. The red dots, representing the loaded data points, should appear in a reasonably shaped curve.
8. Click **Optimize Lines**.
9. Review each cluster again. Ensure that the blue line representing the cluster centers and the dotted gray lines representing the transform windows are reasonable, and pass through or close to the red data points.
10. When you are satisfied with the proposed fit, click **Accept**.
11. Save the compound file. If you would like to review the sample pH sensitive signature for valine, do not replace the file called `Valine-pH.xcpd`.

Chapter 8. Library Manager

8.1 Introduction

You can use the Library Manager module to add new compounds to your library, remove existing compounds, and create and edit compound sets using the compounds in your library. Sources of compounds that you can add to your library include the Chenomx Reference Compounds, compound files (`.xcpd`) created in Compound Builder, compound packs (`.pack`) from Chenomx or from other users of Chenomx NMR Suite, embedded libraries in legacy spectrum files (`.cnx`) and legacy Chenomx library files (`.cnxlib`).

8.2 Adding Chenomx Reference Compounds

Chenomx reference compounds are available for 400-800 MHz NMR spectra. In order to use the reference compounds to profile your spectra, you need to add them to your library.

Adding Reference Compounds to Your Library

1. Open **Library Manager**.
2. Click **Library > Add Compounds...**
3. Select Chenomx Reference Compounds.
4. Click **Next**.
5. Click the checkboxes next to the spectrometer frequencies that you will be using.
6. Click **Finish**.

A Compound Set will be created automatically for each reference library that you install. Note that the Compound Set names also include the version number of the library. It is recommended that you use the same library version for profiling all spectra in a given study.

If required, previous versions of the Chenomx Reference Libraries can be downloaded from our website at http://www.chenomx.com/compounds . A version history is also available there.

8.3 Adding Custom Compounds

To use compound signatures that you create using Compound Builder, you need to add them to your library. Adding compound files (`.xcpd`) to your library makes those compounds available to all modules, so you can use them in Profiler, add them to compound sets, and generally use them alongside the standard Chenomx reference compounds.

Adding Valine and Proline to Your Library

1. Open **Library Manager**.
2. Click **Library > Add Compounds...**
3. Select Importable Compounds, then click the Browse button (...) and navigate to the folder in which you saved the compound files that you created during the Compound Builder tutorial.

If you have not completed the Compound Builder tutorial, you can use the provided sample files in the `Compound Builder` folder.

4. Select your compound files and click **Choose Files**.

   If you are using the sample files, select `Valine-pH.xcpd` and `Proline.xcpd` and click **Choose Files**.

5. Click **Next**.

6. Click the **Track imported compounds in a new compound set:** check box, and enter a name for the new Compound Set that you would like the compound to appear in.

   Or, if you don't want to create a new Compound Set containing the compounds that you're about to add to your library, leave the check box unchecked.

7. Click **Finish**, and click **OK** in the resulting information dialog.

   If you created a Compound Set, it appears in the Compound Sets list. Otherwise, you can find your newly added compounds in the **Compounds** set at the top of the Compound Sets list.

You can use the same method to install compound packs that you've downloaded from the Chenomx website or received from other Chenomx NMR Suite users. Simply select one or more compound pack files (`.pack`) instead of selecting individual compound files. You can even select a folder containing a mixture of compound files (`.xcpd`), compound packs (`.pack`) and legacy files containing library information (`.cnx` and `.cnxlib`) to import from a variety of sources at the same time.

If you've added custom compounds to your library, you may also wish to add your own custom reference cards. These reference cards can be displayed alongside your compounds as you work with them. For more information on creating and installing custom reference cards, see "Reference Card Editor" in the Chenomx NMR Suite User Guide.