Starting NanoAnalyze

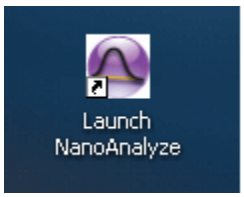


Figure 1 NanoAnalyze desktop icon.

The program opens a window on the desktop.

Figure 2 NanoAnalyze window.

1 The following functions are located on the **Menu** bar:

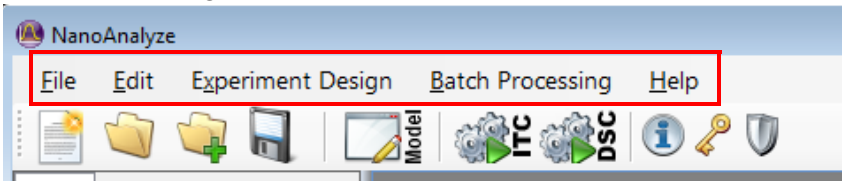


Figure 3 Menu bar.

- **File**: Provides access to file and method operations
- **Edit**: Provides access to the Model Editor, as well as other editing functions
- **View**: Shows or hides the file tree on the left side of the program window
- **Experiment Design**: Opens an experiment planning tool for ITC titrations
- **Batch Processing:**
- **Help**: Access to Help files and program information

2 The following functions are located on the toolbar:

- **New File**: Opens a new file page in NanoAnalyze
- **Open a File**: Opens an already-existing data analysis file (***.csc** or raw data file)
- **Add a File**: Imports an additional raw data file into the file tree
- **Save File**: Saves the current state of NanoAnalyze as a ***.csc** file

- **Model Manager**: Opens a window for managing all model fitting files
- **ITC Batch File**:
- **DSC Batch File**:

The Guardian option provides compliance with 21 CFR Part 11. Detailed information is available in the document “Guardian for Microcalorimetry Software.”

Guardian for Microcalorimetry functions:

- **Show/Hide Notifications**
- **Manage Guardian Keys**
- **Guardian Options**

Working with Nano ITC Raw Data Files

The following describes how to open, view, analyze, and print data with Nano ITC raw data files.

Opening a File

1 Select **Open** in the **File** menu.

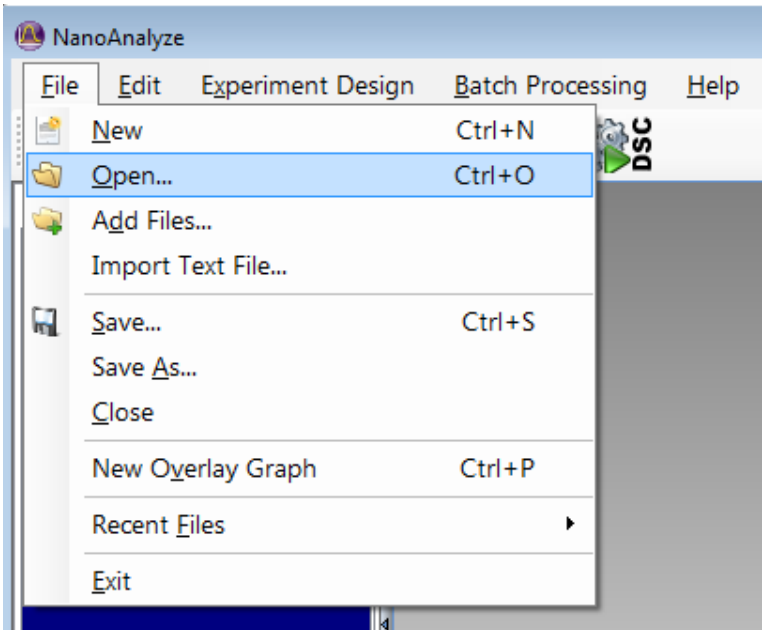


Figure 4 File > Open.

2 Use the drop-down list to select the folder that contains the ITC data files.

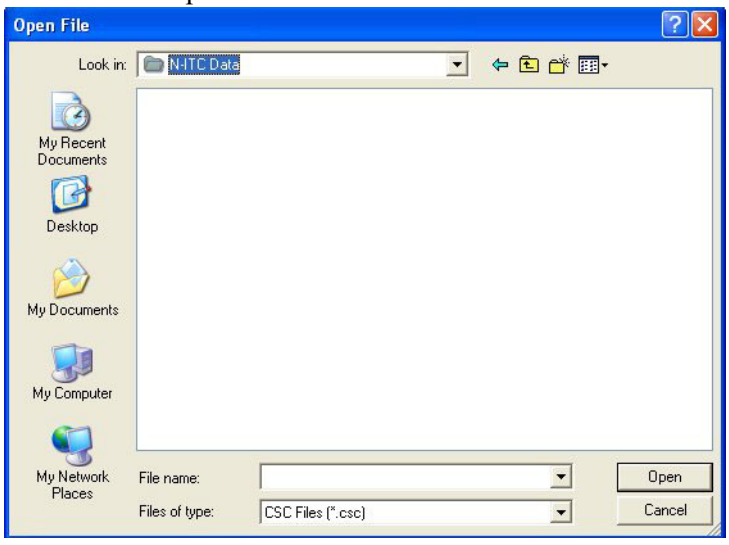


Figure 5 Select folder containing ITC data files.

3 Select data file type **ITC Files (*.nitc or*.jet)** from the drop-down list at the bottom of the window.

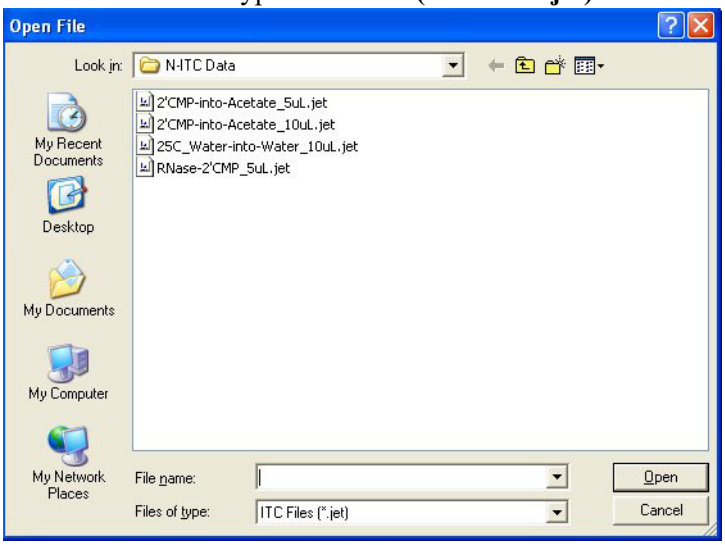


Figure 6 Select data file type.

4 Select the experiment data file that you wish to load, then click **Open**.

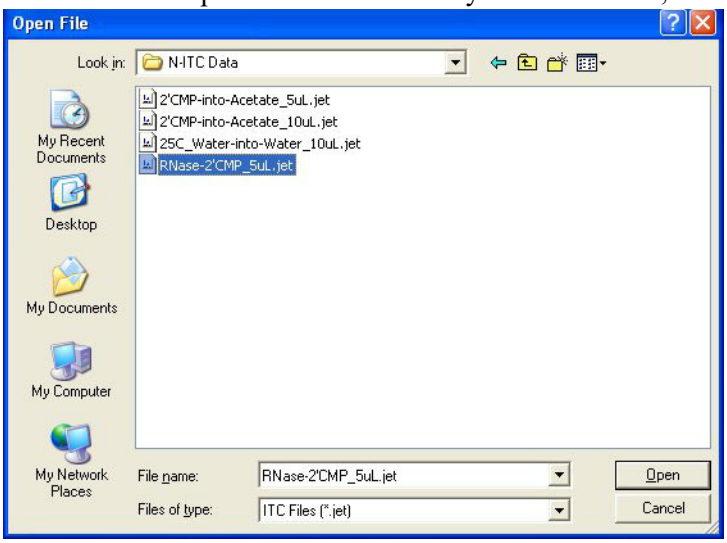


Figure 7 Select experiment data.

NOTE: Multiple files can be loaded at the same time if desired. Select the first file by clicking on the name with the mouse. Select a range by holding down the Shift key while clicking on the last file, or select any number of individual files in any order by holding down the **Ctrl** key while clicking the files with the mouse.

5 A blank experiment can be performed along with the titration. Blanks include the heat effects that result from fluid motion during the injection, along with enthalpies from dilution.Typically the syringe is loaded again with the syringe reactant; however, the sample cell is filled with the buffer that was used to dialyze the sample. Select **Add file..** from the file menu and select the blank file to be loaded. You will reference this blank experiment data file in a later step.

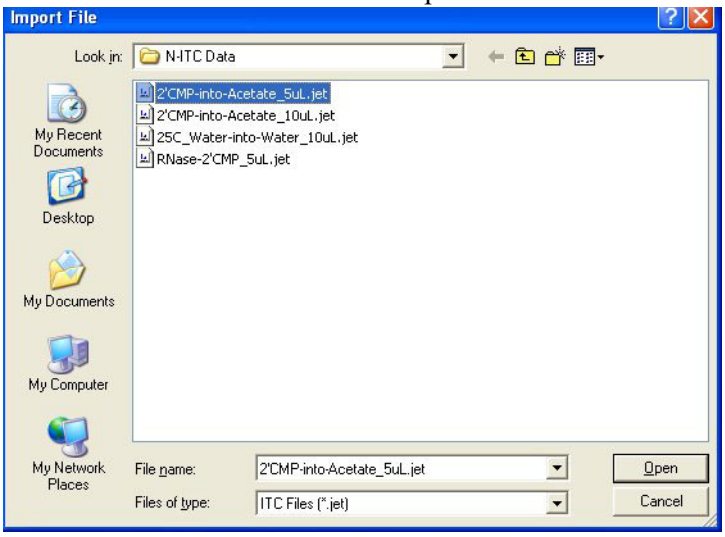


Figure 8 Select a blank experiment.

6 Click on the titration experiment file in the file tree to load the file for analysis. The data file appears in the file tree and a table of the measured heat data appears in the **Data Columns** tab on the right side of the program window. The figure below shows the appearance of the file tree after both the sample and blank data files have been brought into NanoAnalyze.

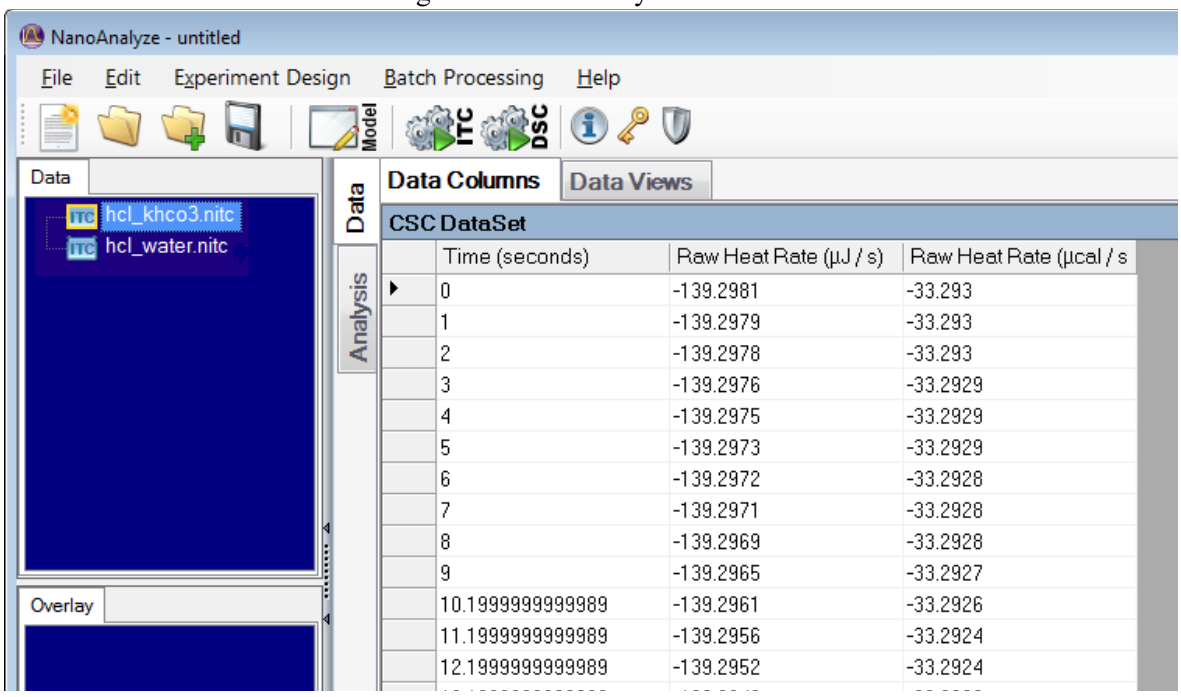


Figure 9 Data Columns.

Analyzing ITC Data

To access data analysis functions, click on the **Analysis** tab.

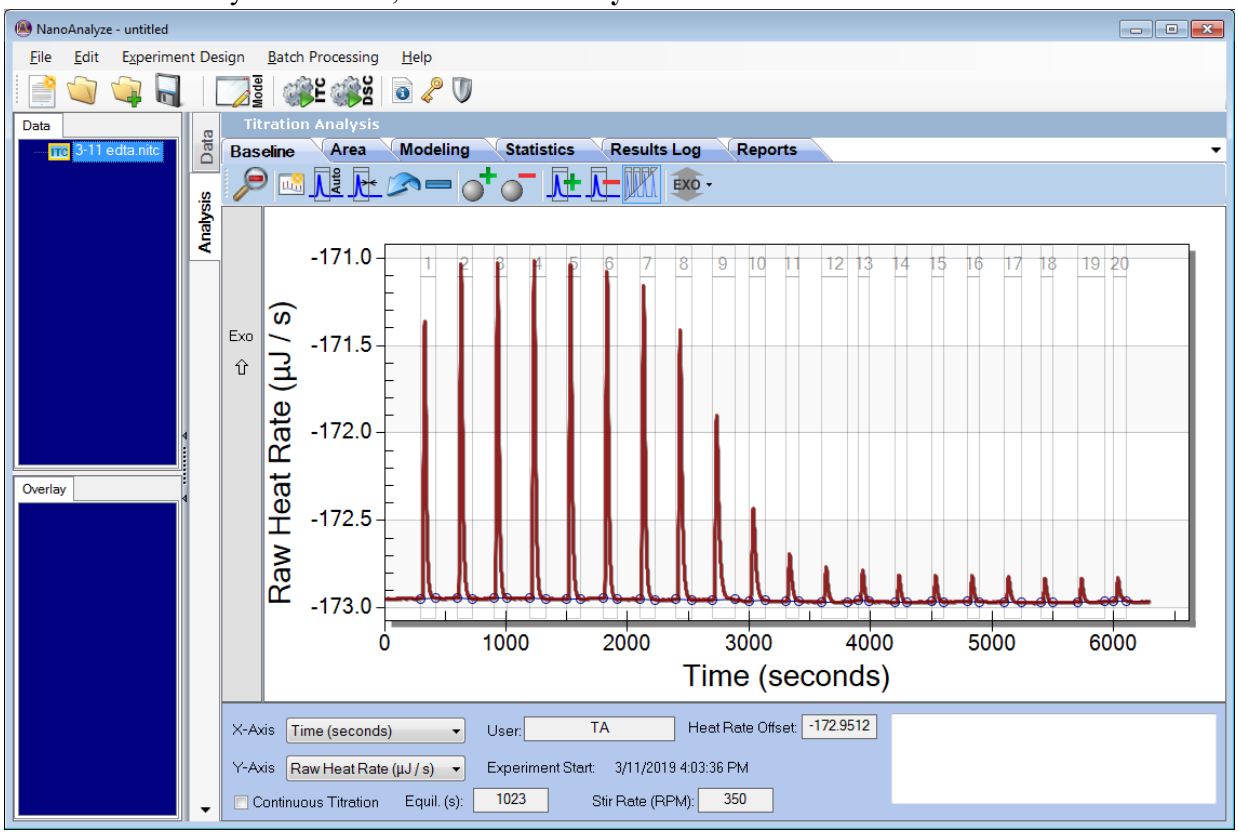


Figure 10 Analysis.

Selecting Integration Regions

Select integration regions automatically by entering the injection time table parameters.

1 Select **New baseline** by clicking the button in the toolbar in the **Analysis** tab.

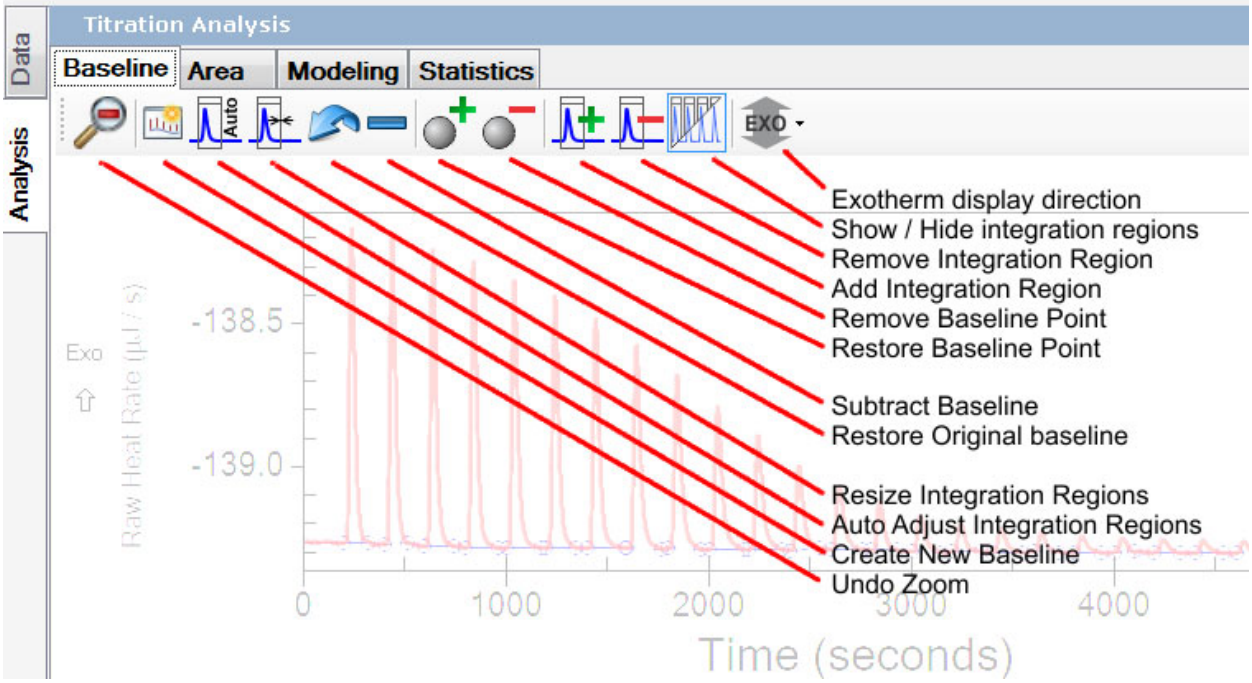


Figure 11 New Baseline.

The following are the available toolbar functions:

- **Undo Zoom**: Restores a zoomed-in view to show all of the experimental data
- **New Baseline**: Sets the integration window start times and width

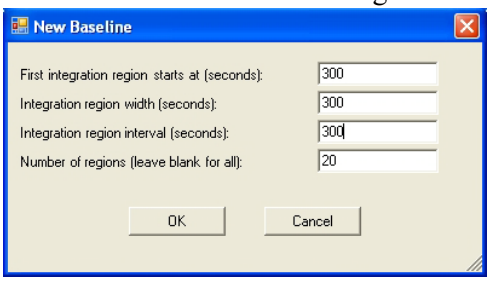


Figure 12 New Baseline dialog box.

- **Auto Adjust Integration Regions**: Integration start and stop times and the baseline nodes are automatically adjusted according to signal characteristics. This function can be set as the default behavior when an ITC data file is brought into NanoAnalyze. In the Edit menu the “Default ITC Settings” option displays a dialog box that contains a checkbox to enable the auto baseline feature as well as a selection menu for the default enthalpy units.

- **Resize Integration Regions**: This manually sets all integration windows to one common width. Individual windows can be adjusted as needed by clicking and dragging the right edge of the window border.
- **Restore Original Baseline**: Reset the baseline and integration regions according to default settings
- **Subtract Baseline**: Subtracts the baseline from the signal data, and assigns the value of 0 microWatts to the baseline
- **Restore Baseline Point**
- **Remove Baseline Point**
- **Add** and **Delete Baseline Pivot Points**: Adjusts the path of the baseline
- **Add** and **Subtract Integration Regions**: Adjusts the time intervals of the integration regions
- **Show/Hide Integration Regions**: Toggles between the two functions
- **Set Exotherm Display Direction**

2 Enter the following:

- Time of the first injection
- The desired width of each integration region (as a first approximation this can be set to the injection interval)
- The injection interval
- The number of injections that occurred (or leave this field blank)

3 The experiment data divides into the integration regions.

Narrow the integration regions by entering a smaller number for the integration region width. Typically the heat signal rises from the baseline at some time after the actual injection start time. Adding a few seconds to the start time aligns the integration window to the measured heat signal.

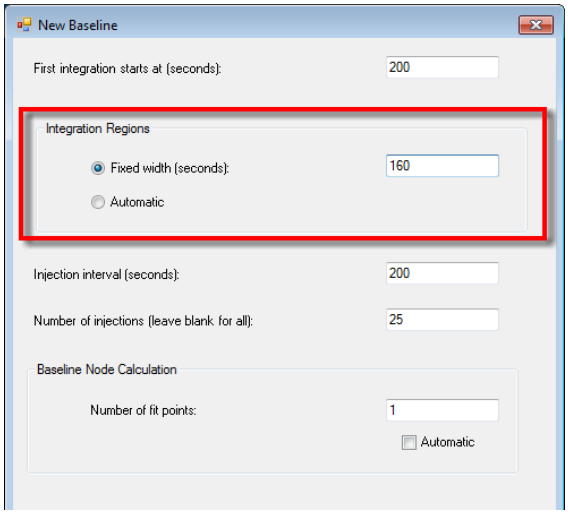


Figure 13 Integration width.

The graph now shows the narrower integration regions.

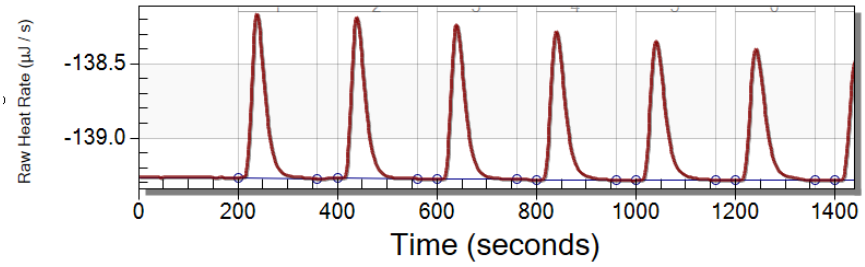


Figure 14 Baseline graph.

Examine the baseline in detail by zooming in on a selected portion of the graph.

4 Press and hold the left mouse button at one corner of the zoom region, move the mouse to define the opposite corner, and then release. The graph is redrawn to the selected region.

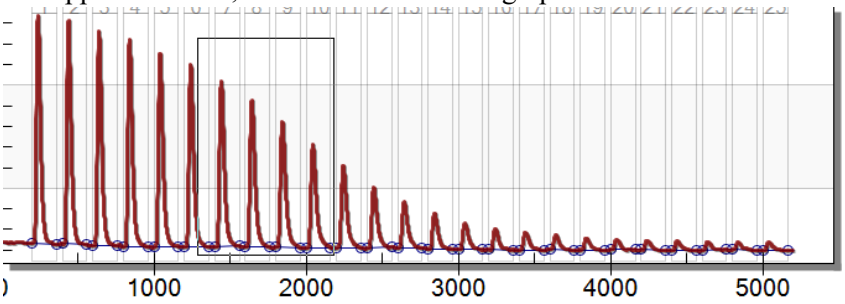


Figure 15 Baseline graph.

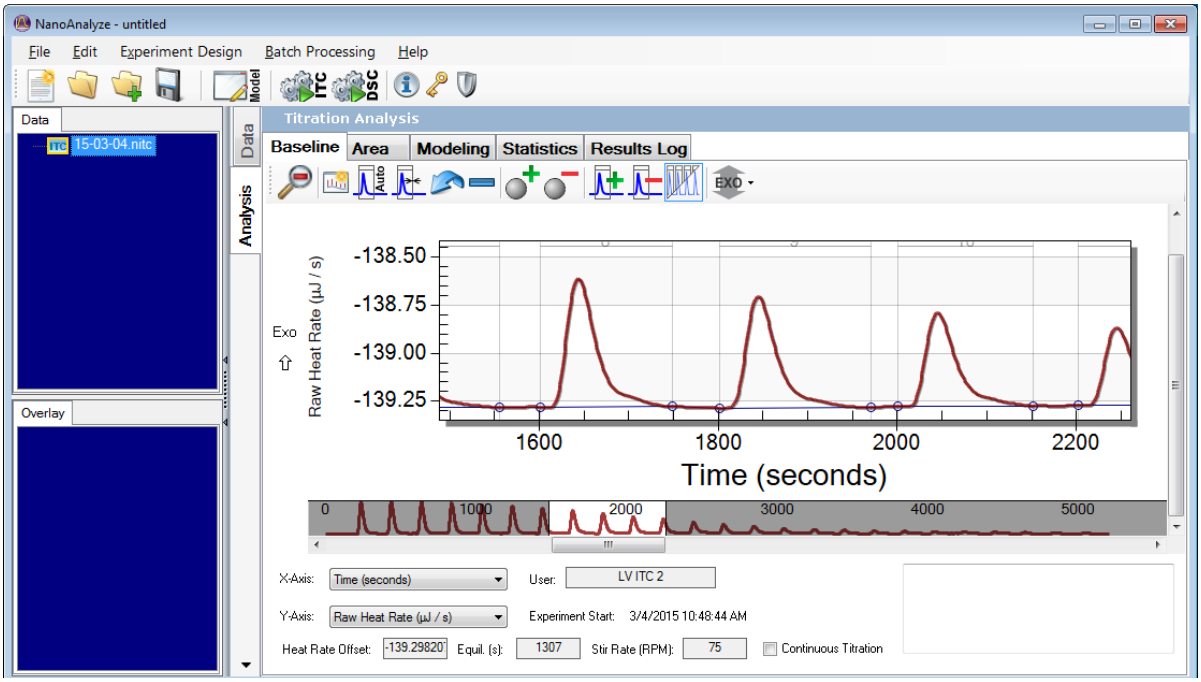


Figure 16 Baseline graph.

Integration regions can be adjusted individually:

1 Magnify the baseline region of interest, if necessary.

2 Adjust baseline pivot points if desired by moving the mouse cursor over the blue circles, then pressing and holding the left mouse button. Move the circle to the desired new position and release the button. Control handles for the start and end times are at the tops of the vertical gray lines. Adjust these times by clicking and dragging with the mouse.

The buttons above the data graph window provide additional controls for adjusting the integration regions as well as the appearance of the data display. The baseline pivot points may happen to fall on the peaks or valleys of the baseline noise. For the best results, the baseline should run through the middle of the noise band, and the integration windows should be just wide enough to include the peak without any additional baseline. The experiment data can be examined at high magnification by first zooming in, then scrolling left to right using the mouse wheel. The integration windows can be adjusted if necessary by clicking and dragging their starting and ending boundaries. The baseline can be adjusted by moving, adding, or deleting the pivot points.

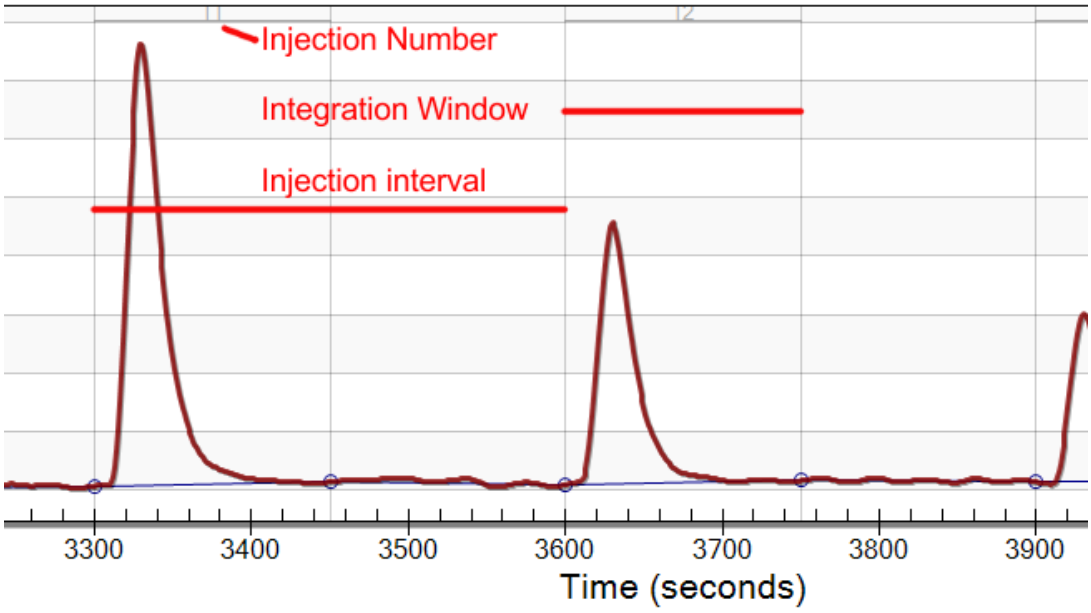


Figure 17 Baseline graph.

Integration regions are bounded vertically (heat rate axis) by the signal trace and the baseline, and bounded horizontally (time axis) by the start and end markers. Areas above the baseline are negative (heat was liberated from the sample). Areas below the baseline are positive (heat was absorbed by the sample).

3 Select **Undo Zoom** to review the graph for any additional modifications to the integration regions or the baseline fit. Alternatively, navigate along the baseline graph by rolling the mouse wheel (if the mouse is so equipped).

Peak to Width Ratio

On Affinity ITC instruments with serial numbers 25001 and above, experiment data is stored in data files as raw values that have not yet been processed for smoothing. NanoAnalyze software features three levels of smoothing that are applied after the experiment data has been collected. The LOESS method of data smoothing is employed, which has the following advantages vs. an exponential filter:

- The smoothing is applied symmetrically in both directions on the time axis (no skewing to the trailing side)
- For a given level of baseline noise result, signal events experience less increase of width at the baseline
- Incremental titration experiment injections can be run at shorter time intervals, significantly decreasing the total time required for a complete experiment
- The degree of smoothing can be altered for the purpose of signal inspection in order to gain confidence regarding whether an observed feature is only baseline noise that can be excluded from the integration region.

NanoAnalyze never alters the original data records. The degree of smoothing can be changed at any time after a data file is loaded. Select a data file, and then click on **Edit** > **Properties** to bring up a dialog box of data handling controls.

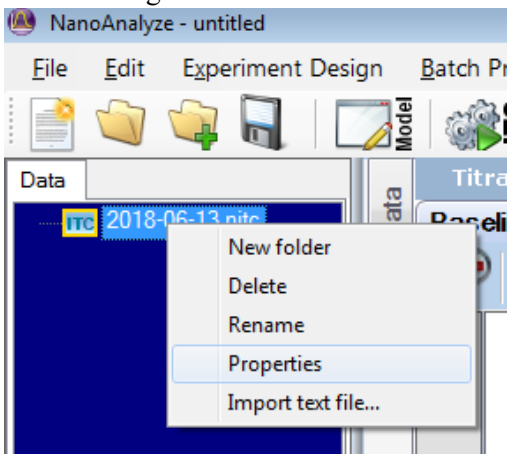


Figure 18 Edit > Properties.

The "Peak to Width Ratio" control sets the degree of smoothing.

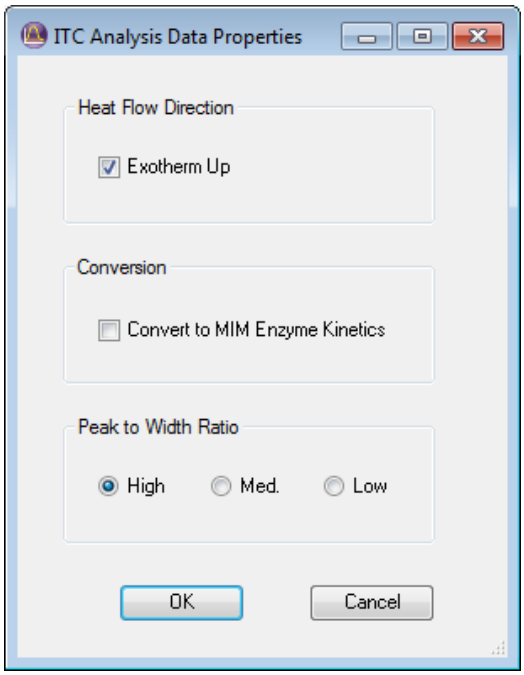


Figure 19 Properties.

Peak/Width Ratio	Peak Height	Approximate Peak Width (Note: Peak widths are also affected by stirring speed)
High	Taller	30 seconds
Medium	Intermediate	45 seconds
Low	Shorter	70 seconds

ITCRun software with compatible Affinity ITC instruments also features the same smoothing selection. When one of these data files is brought into NanoAnalyze, the Peak/Width Ratio is preserved according to the setting that was in place during the experiment in ITCRun.

Figure 20 illustrates the effect of the three different levels of the control. The same data file was loaded three times and each was set for a different level of smoothing. The vertical placements were manually offset to make visible the baseline noise characters.

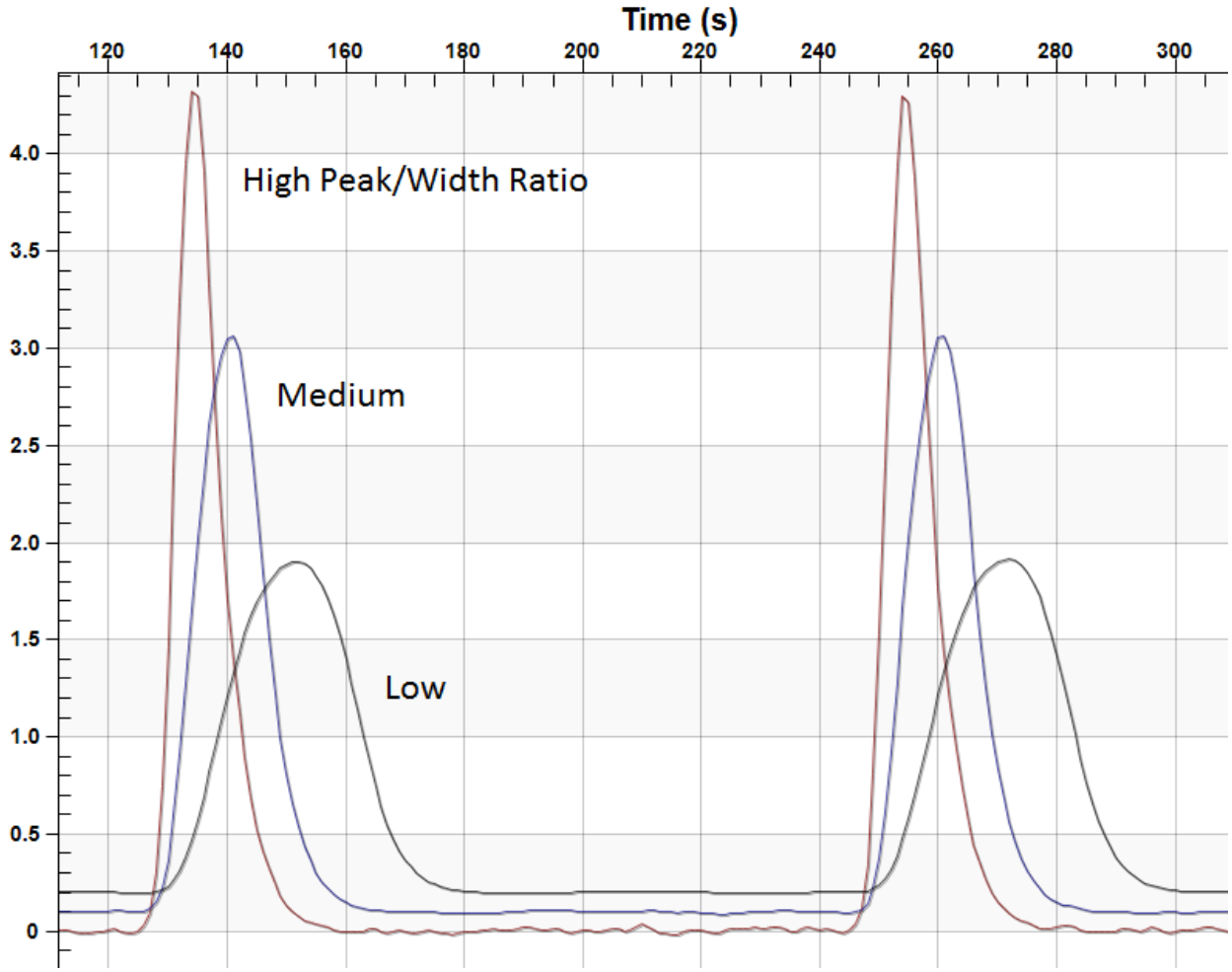


Figure 20 Effect of Peak/Width Ratio Control.

Figure 21 shows the same set of three differently-set copies of the identical data file after analysis. The appearance of the data in the three versions differ but the integrated peak areas do not vary by significant amounts. The titration curves therefore also do not vary to a significant degree as long as baseline resolution of the peaks is maintained.

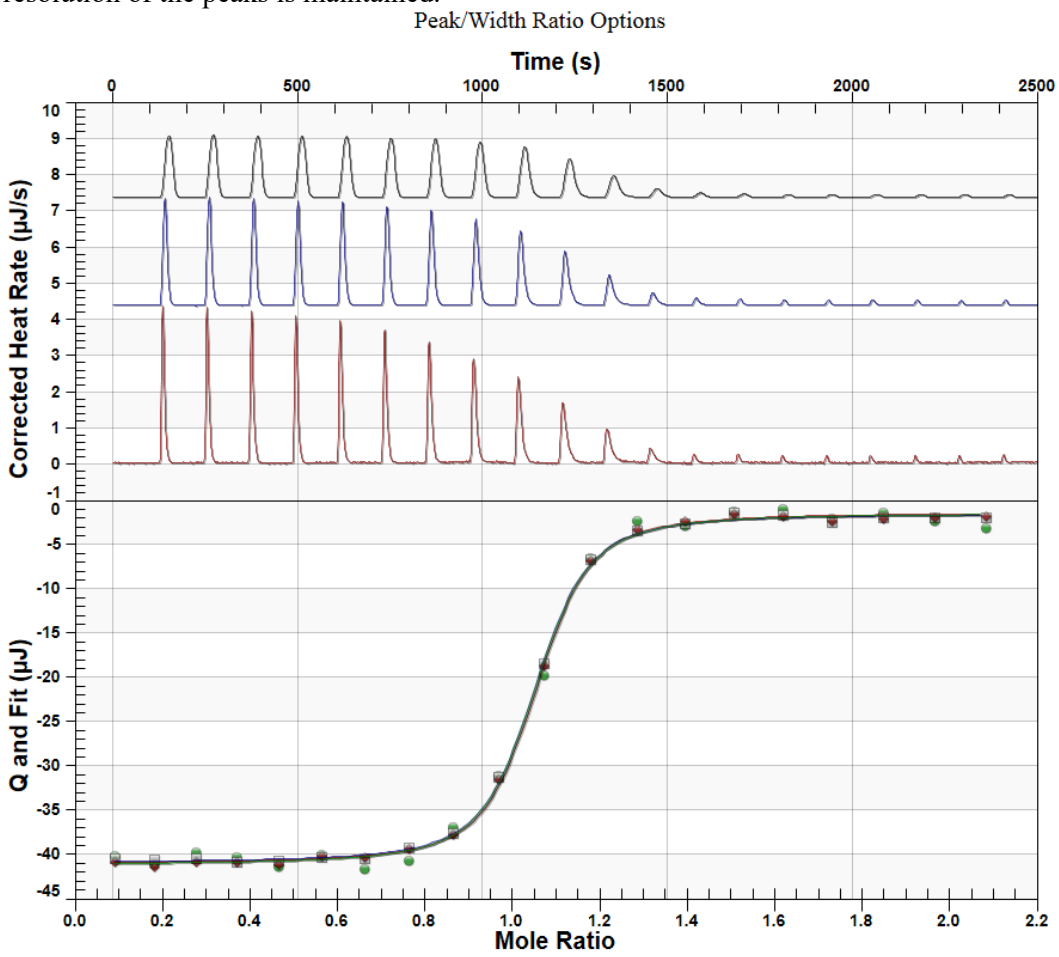


Figure 21 Invariance of analysis results at various smoothing options.

The matching setting in ITCRun software is accessed via the **Settings** > **Advanced** controls tab. The setting in use at the time of the experiment is saved inside the data file.

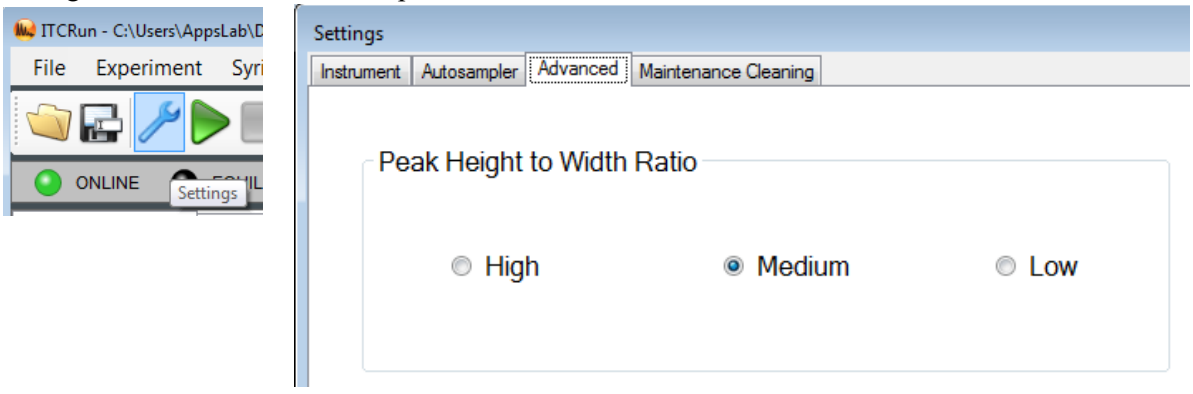


Figure 22 **Settings** > **Advanced**.

Entering the Titration Data

The sample concentration data and cell volumes are automatically imported when loading a data file that was collected in ITCRun Version 1.7 or above, or DSCRun Version 3.6 and above. With data files that were collected using older program versions, enter the experimental data manually as follows (ITC example shown):

1 Select the **Area** tab and enter the reagent concentrations. **Syringe Concentration** is the concentration of the reagent that was injected during the experiment.

Enter the concentration of the reagent that was loaded into the reaction cell in the **Cell Concentration** data entry box.

2 Enter the sample cell volume of the Nano ITC, if not already present.

Analysis of titration experiments requires an accurate figure for the effective cell volume, which varies slightly from one instrument to another. For best results, a chemical calibration should be performed to establish this figure. Detailed procedures are documented in "Calibration of nanowatt isothermal titration calorimeters with overflow reaction vessels", Neil A. Demarse, Colette F. Quinn, Dennis L. Eggett, Donald J. Russell, Lee D. Hansen, Analytical Biochemistry 417 (2011) 247-255.

If a chemical calibration of the cell volume has not yet been performed with a specific instrument, these default volumes will be fairly close:

Cell Type	Affinity ITC	Nano ITC
Standard Volume	965 microLiters	950 microLiters
Low Volume	185 microLiters	170 microLiters

Once the cell volume has been established, enter the figure in the provided entry box in the Settings tab of ITCRun. The figure will be saved in the non-volatile memory of the instrument.

NOTE: While not ideal, if available sample volumes happen to be severely restricted it is possible to perform an experiment with the sample cell only partially filled at the start of the experiment. If this is done, the sample volume loaded into the cell plus the reagent volume in the syringe must be less than the sample cell volume. No fluid will be displaced from the sample cell during the experiment. In these cases, check the **Partially Filled Cell** box and enter the volume of reactant mixture that was loaded into the cell.

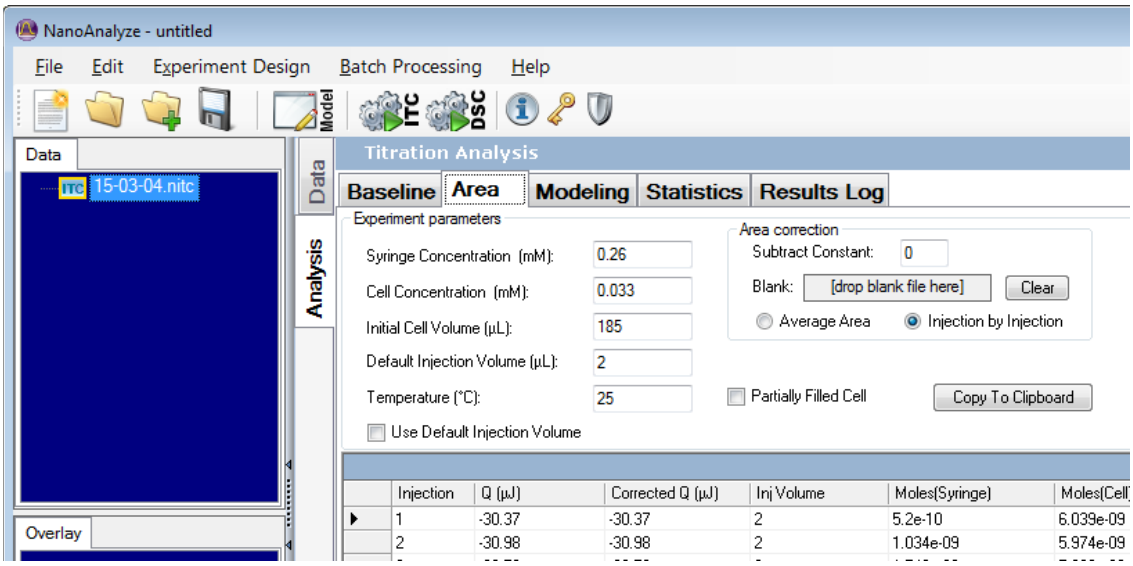


Figure 23 Area tab.

Subtracting the Blank Experiment

1 Select the blank experiment run from the file tree by moving the cursor onto the file name and holding down the left mouse button.

2 Drag the file over to the box labeled **Blank (drop blank file here)**.

3 If the blank peaks are small, they may also be inconsistent in area. In this case, select the **Average Area** option. Otherwise, when blank peaks are a substantial fraction of the titration run, this indicates that the heat of dilution may be relatively large. In this case, the peaks decrease in size during the run and the best results may be obtained by selecting the **Injection by Injection** option.

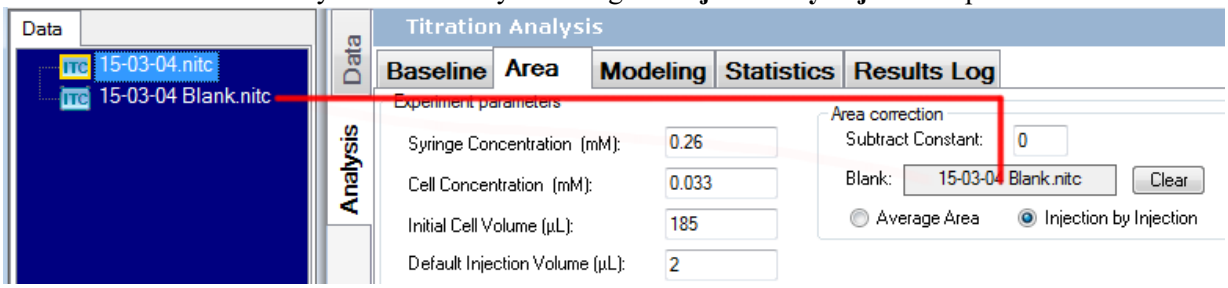


Figure 24 Drag the file to the Blank box.

Hint: Use only one of these methods to enter Blank injection correction values:

- Blank file drop box
- Subtract Constant number entry box
- Blank model (in the **Modeling** tab)

Hint: If you do not have a matching blank experiment, an approximate blank correction can be made if the reaction has essentially reached saturation, meaning that the final peaks are small and have a flat trend (they are gradually approaching a limiting value). The final injections in the sample experiment may be effectively the same as blanks if the reaction has gone to completion; in some cases they can also be used to approximate the blanks of every injection in the experiment. This method applies a constant value from the **Subtract Constant** (below **Area Correction**) window to every injection in the experiment. Be sure to match the sign of Q as seen in the injection table. Enter an estimate of the final convergence value into the box labeled **Subtract Constant**.

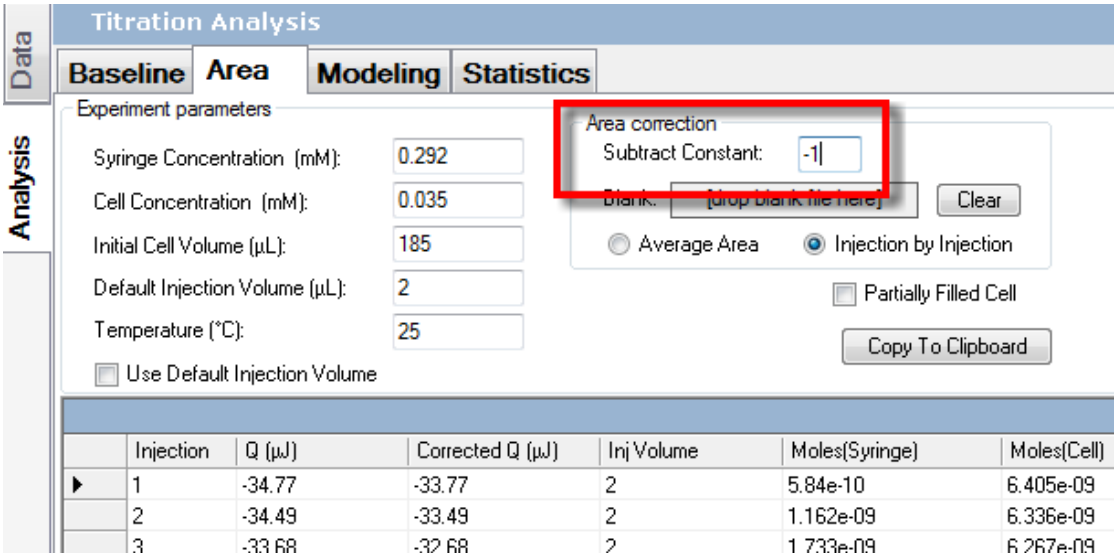


Figure 25 Subtract Constant.

Fitting a Model to Titration Data

Exporting a Previously-Fitted Model to the New Version of NanoAnalyze

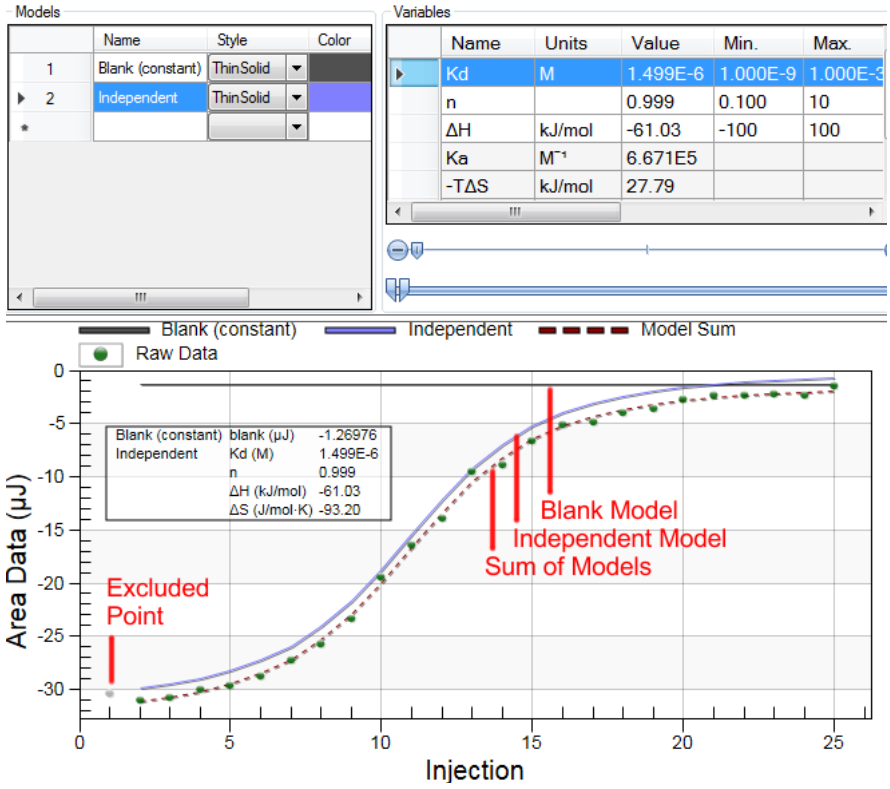
NOTE: A new database for the data-fitting models was introduced in NanoAnalyze version 1.2. If you have created or modified any models while using versions prior to 1.2, please follow these instructions to ensure they remain available for use with versions 1.2 or higher. Perform these steps before installing the new version.

1. Open the existing version of NanoAnalyze and select **Edit** > **Model Editor** from the menu.
2. In the model directory tree, right-click on the customized model you wish to preserve.
3. Select **Export model...** and save the file to a directory of your choice. After exporting all desired customized models, exit NanoAnalyze and uninstall the software from your system.
4. Install the new version of NanoAnalyze and start the software. Select **Edit** > **Model Editor** from the menu.
5. Right-click the **Models** folder and select **Import Model...** From the browser window, select the model to be imported. Repeat this step for all the models you wish to import.

Fitting a Model Using the New Version of NanoAnalyze

1 Select the **Model** tab.

The data points in the graph correspond to the integrated areas of the injection peaks. The first peak is typically smaller than the following peaks due to a smaller amount of reagent injected at the first interval. (A common practice to prevent reactions from occurring during the thermal stabilizing before the experiment is to briefly blot the tip of the syringe with a clean tissue. This introduces an air bubble which acts a as a physical barrier to prevent contact between the reagents in the cell and the syringe barrel.)



Hint: If you are including a blank model, be sure that no Blank injection corrections were added in the **Area** tab.

Figure 26 Model tab.

2 Select a model to be used to fit the titration data.

In cases where the binding model is unknown, it may be necessary to attempt to fit according to more than one reaction model.

3 Click the control labeled **Model** to bring up a list of the available models that have been stored in the program.

4 Click the + to show the list of available models. Multiple models may be selected in this window; a typical use for this is to combine the Independent model with one of the Blank models. Click **OK** when you have selected the desired model or models.

Note that some combinations of models can have matching degrees of freedom. One wouldn't normally combine the Blank (constant) along with the Blank (linear) model; both contain a constant

term and the two models would share this in an unpredictable way. The sum of the fits, however, would be mathematically valid.

Hint: In some cases, having too many degrees of freedom can slow down the fitting process. When this happens, several things can help:

- Use the slider controls to obtain an approximate fit
- Temporarily disable some variables from the fitting process
- Adjust the limits of the acceptable ranges of the variables

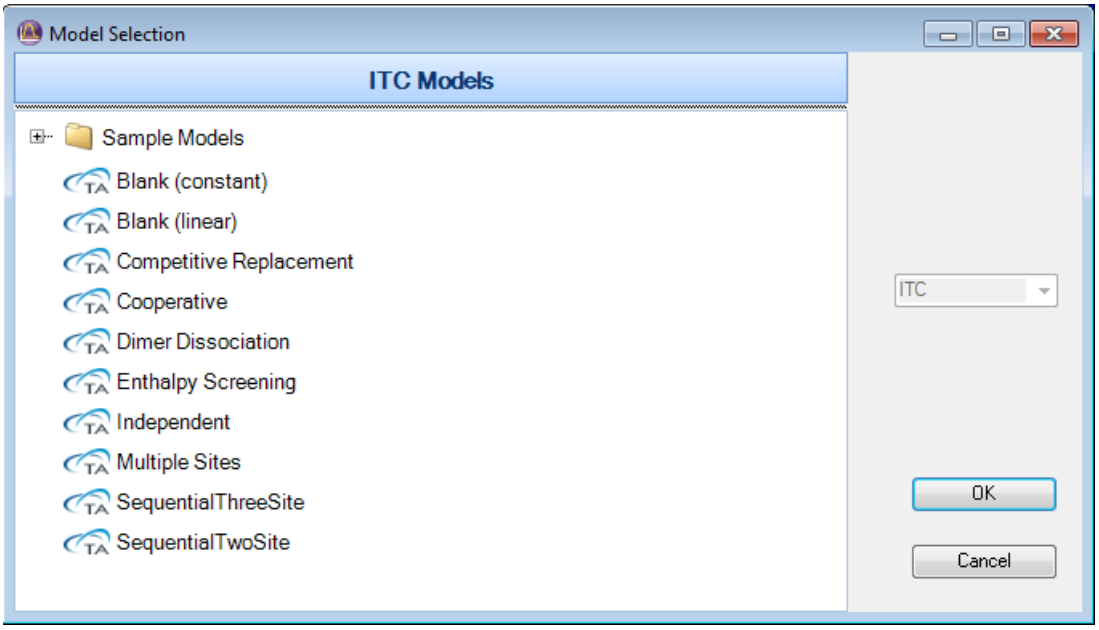


Figure 27 List of available ITC models.

Initial values for the fit parameters are listed above the mathematical description of the model.

5 Choose the desired fitting model, then click **Select** to proceed.

6 The toolbar items allow you to perform the following:

1 Start Fitting the model(s)
2 Stop the Fitting process
3 Fit Settings
4 Variable Constraint (narrows or widens the searchable range)
5 Fit Tolerance (narrower = more precise, but also more time)
6 Stop the Fit when any Local Minimum is found
7 Exclude the 1st Data Point from the Displayed Curve (also see item 10)
8 X-Axis Option: toggles between Injection Number and Molar Ratio
9 Full Screen display mode
10 Exclude data Points from the Curve Fit
11 Include Data Points in the Curve Fit
12 Export Data or Image to the Clipboard
13 Export Data or Image to a File
14 Print the Page
15 Create Overlay Graph
16 Save the Current Settings as the Default for thisPage
17 Reset the Graph Settings as the Default for this Page
18 Optimize Concentrations (ITC: launches the Experiment Design explorer using Current Values of Sample Concentrations and Model Fit Parameters)

Figure 28 Toolbar options.

Removing Outlier Data

1 Move the mouse cursor over any data point that will not be included in the model fitting. The cursor changes into a small crosshair when the cursor is placed correctly.

2 Click the left mouse button to deselect the data point. Deselected points appear as gray dots.

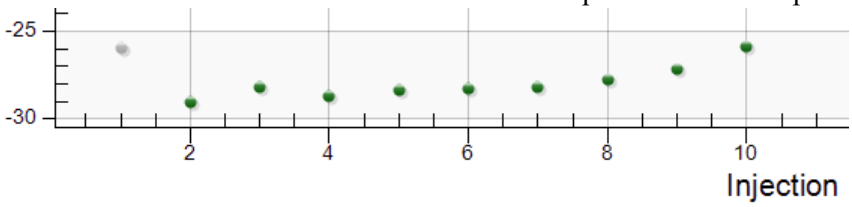


Figure 29 Deselected point.

Starting a Fit

Click **Start Fit** to begin the fitting process. The fit process runs a series of attempts, each of which improves the fit. In some cases, the time limit or the maximum permitted number of attempts expires before the fit is optimized. If this happens, simply click **Start Fit** again.

Adjusting the Model Fitting Behavior

The model is an equation used to describe the experimental data curve. At the bottom of the **Model** tab is a graph displaying the residuals (the differences between the data points and the model equation). The purpose of the model is to fit the equation to the data in such a way that the sum of the residuals is minimized, meaning that the equation curve follows the data points closely. The equation parameters are the thermodynamic variables that have been determined.

Quite often, the fitting algorithm will take a path where eventually the minimization cannot improve. This is considered a local minimum; the best possible fit is the global minimum. There is no guarantee that the first minimum found will be the global; therefore successive attempts will often deliver a better fit. NanoAnalyze software will automatically run several cycles of attempts. At any time that the cycling process halts, the best fit discovered so far will display, even if it was discovered in a previous cycle.

After the model has converged on a preliminary solution, the software runs through successive cycles of exploration of the parameters. The parameters are adjusted by a random amount, and then the fitting process starts again automatically.

1 To adjust the model fitting behavior, use the controls described below:

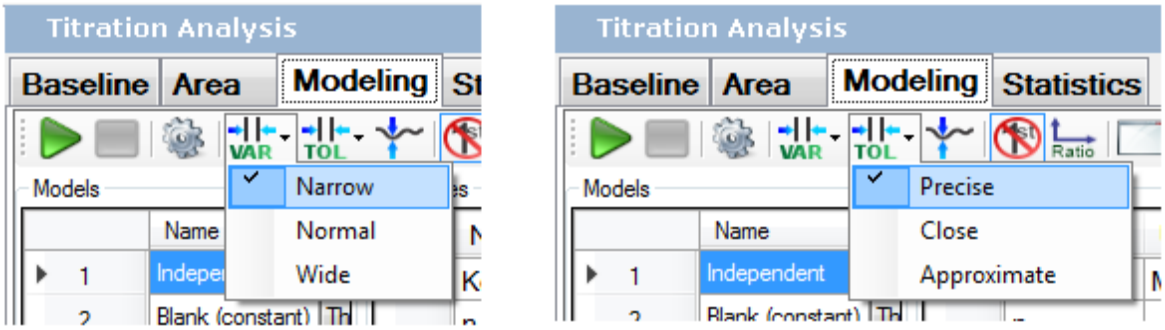


Figure 30 Adjust model fitting behavior.

- The drop-down menu labeled **Variable Constraint** controls how far the parameters randomly adjust on each cycle. The **Narrow** setting restricts parameter changes between cycles to small increments, while **Wide** allows for larger changes. Sometimes a wider change is required in order to break out of a local optimum of the fit. A change of the setting takes effect when the fit is started and when a new cycle begins after a local minimum is found.
- **Fit Tolerance** controls the magnitude of the acceptance threshold. When the threshold is met, the fitting process halts. Selecting **Precise** restricts the fitting process to complete only when the quality of the fit to the data is extremely close, or when the process times out–whichever comes first. The **Approximate** setting allows for more rapid conclusion to the fit process at the possible expense of the ultimate quality of the fit.
- The **Stop at First Minimum** icon on the toolbar will stop the cycling after the next local minimum is found. This check box and the two menu selections above can be adjusted at any time, even if the model is running.

2 Press **Start Fit** to proceed.

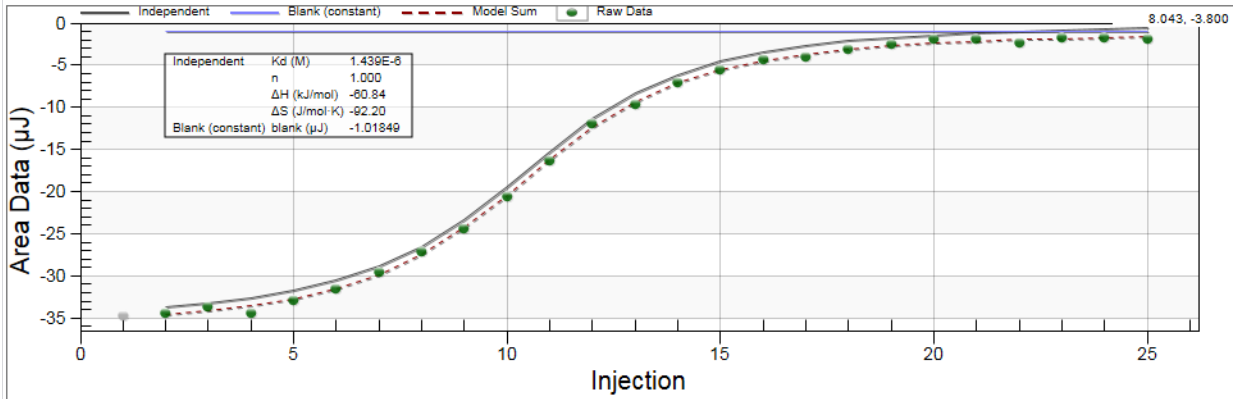


Figure 31 Start Fit.

3 After the best fit to the data is obtained, some points may be revealed as outliers. These can be deselected and the fit process started again. If the set of data points is altered by selection or de-selection, the memory of the previous best fit is discarded.

The fit parameters K (binding constant), dH (enthalpy), and n (reaction order) are listed in the upper right of the program window and in the parameter table inside the chart area. The chart area can be customized in a wide variety of ways. The parameter table in the chart can be repositioned by clicking and dragging with the mouse. Font sizes and other chart features can be adjusted using the extended controls. Click the bar labeled “Graph Settings” to the right side of the graph area to make the controls available.

Working with Nano DSC Raw Data Files

The following describes how to open, view, analyze, and print data with Nano DSC raw data files.

Opening a Nano DSC Raw Data File

1 Select **Open** in the **File** menu.

2 Use the drop-down list to select the folder that contains the DSC data files.

3 Select data file type **DSC Files (*.dsc)** from the drop-down list at the bottom of the window. Files created in previous versions of DSCRun (***.scn**) can also be opened.

4 Select the experiment data file that you wish to load, then click **Open**.

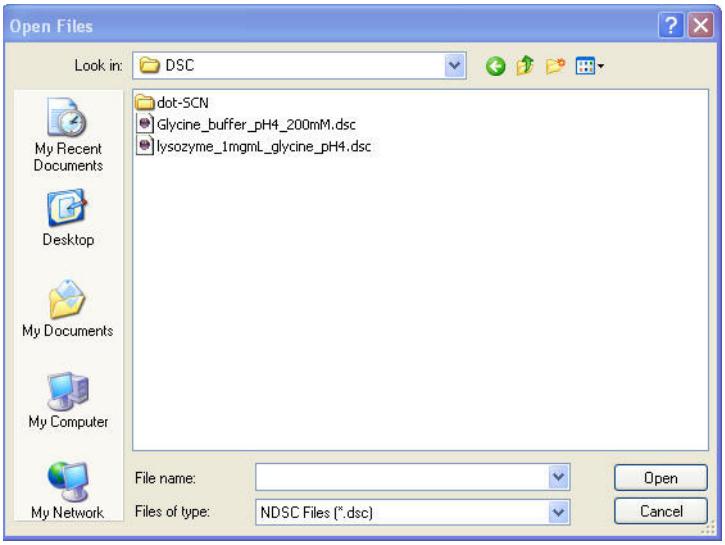


Figure 32 Select experiment data file.

NOTE: Multiple files can be loaded at the same time if desired. Select the first file by clicking on the name with the mouse. Select a range by holding down the **Shift** key while clicking on the last file, or select any number of individual files in any order by holding down the **Ctrl** key while clicking the files with the mouse.

5 When the data file has loaded and appears in the file tree, click on the titration experiment file in the file tree to load the file for analysis. The data file appears in the file tree and a table of the measured heat data appears in the **Data Columns** tab on the right side of the program window.

6 A buffer scan experiment is often performed along with the sample scan. Buffer scans include the heat effects that result from heat flow changes strictly due to an individual buffer. For a buffer scan, typically the sample cell is loaded with the buffer that was used to prepare the sample. Select **Add file..** from the **File** menu or toolbar and select the buffer scan file to be loaded. You will reference this buffer scan experiment data file in a later step.

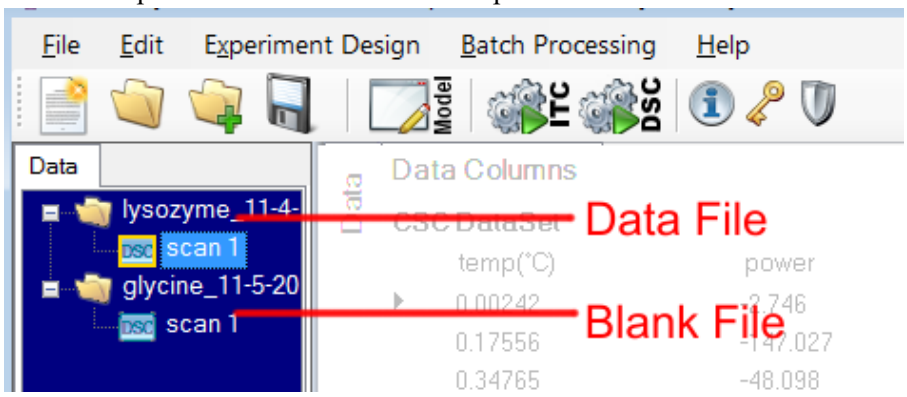


Figure 33 Buffer scan.Analyzing DSC Data

To access data analysis functions, click the **Analysis** tab.

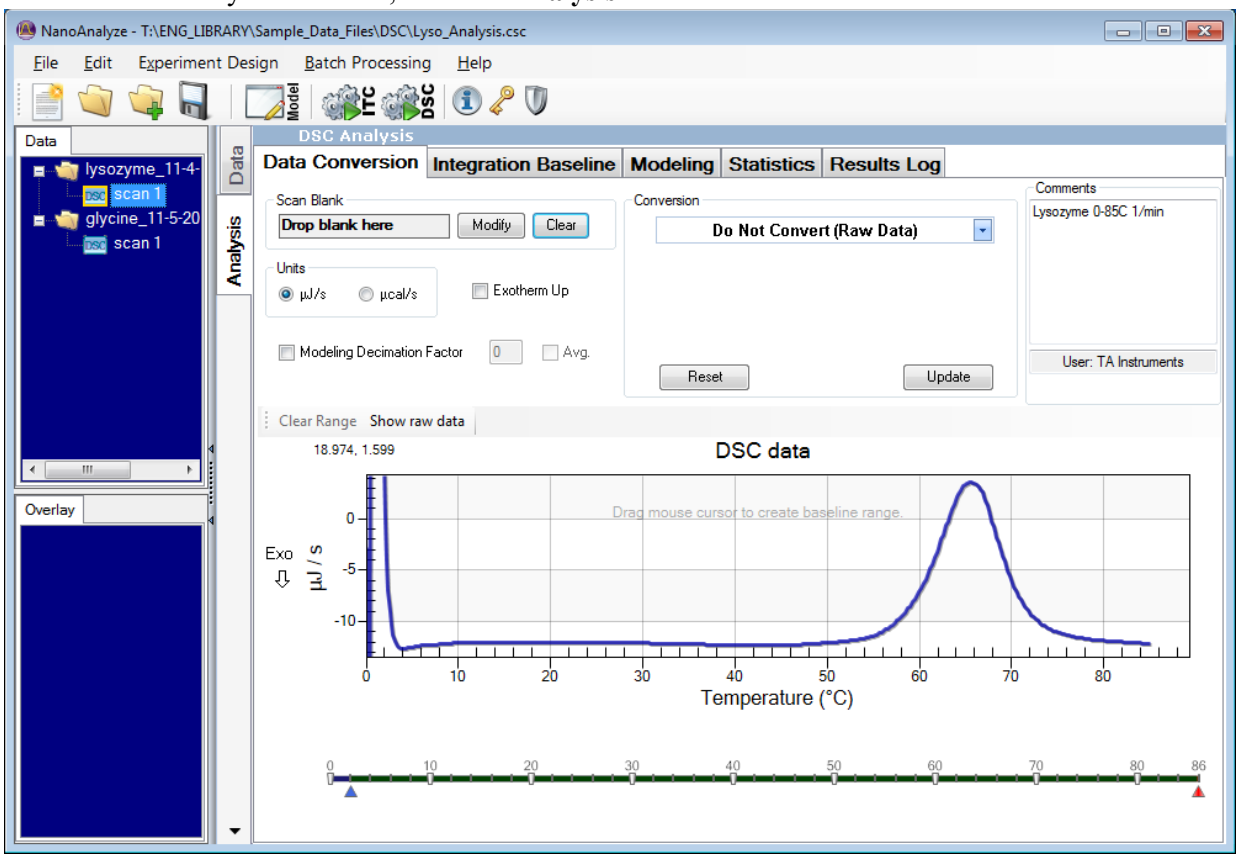


Figure 34 Analysis tab.

Automatically Subtracting the Buffer Scan

To automatically subtract the buffer scan from the sample data, perform the following steps:

1 Select the blank experiment run from the file tree by moving the cursor onto the file name and holding the left mouse button down.

2 Drag the file to the box labeled **Blank (drop blank file here)**.

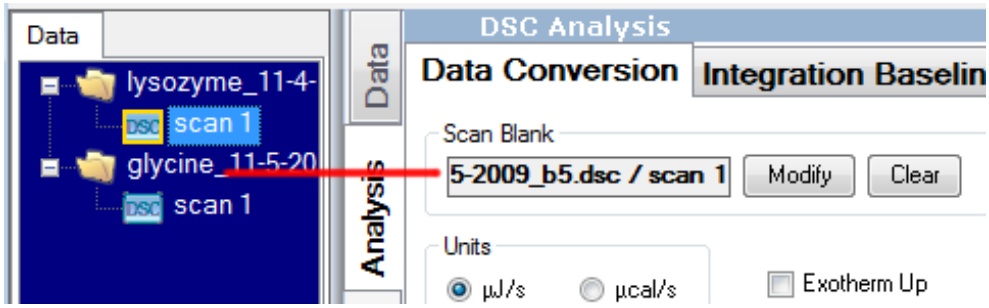


Figure 35 Drag the file to the Blank box.

3 If it has not already been entered, fill in the **Conversion to Molar Heat Capacity** information.

a Enter the volume of the sample cell in the **Volume (ml)** data entry box. This value is typically 0.30 and will always be the same number for an individual instrument.

b Enter the concentration of the sample in the **Concentration (mg/ml)** data entry box.

c Enter the Molecular Weight in kilodaltons in the **MW (KD)** data entry box.

d Click the box preceding the **PSV** (Partial Specific Volume) data entry box. A check mark appears. A PSV value of 0.73 is typical for most proteins in aqueous buffers. Adjust this value as needed for other buffer systems.

e Click the box preceding **Convert to MHC** to place a check mark in the box.

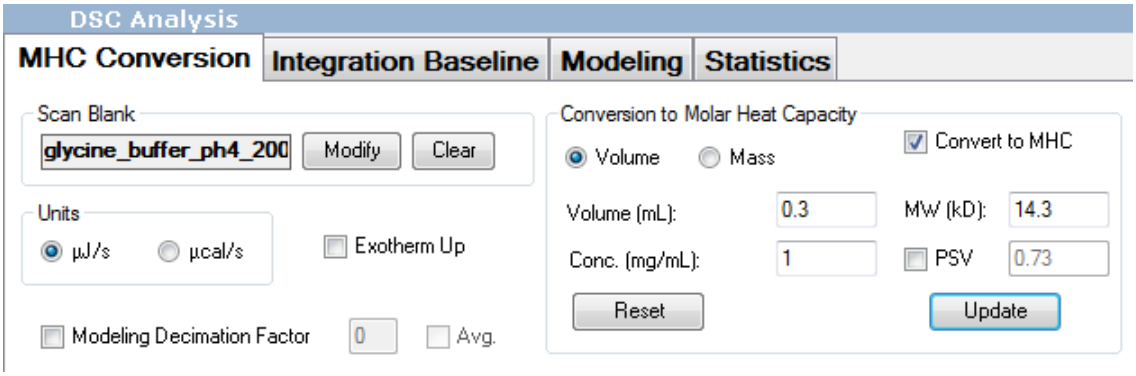


Figure 36 Convert to MHC.

f Click **Update**. The displayed data automatically transforms and the Y scale now displays in **kJ/mol*K**.

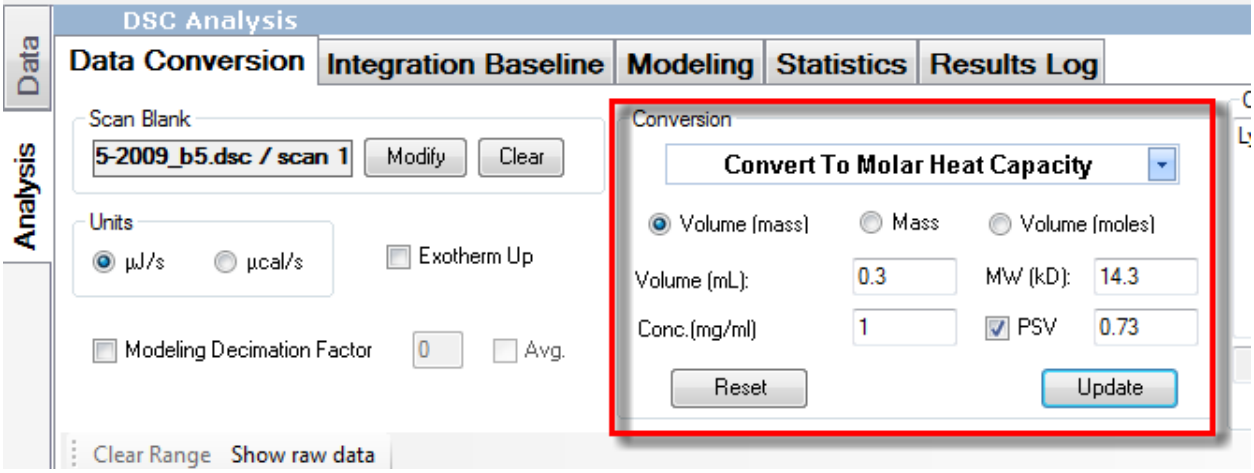


Figure 37 Data entry field group for conversion to molar heat capacity.

Selecting Integration Regions

1 Set the baseline range by moving the cursor into the graph display area. The cursor becomes a plus **(+)**. Place the + on the left of the peak where you want to start the pre-transition baseline, hold down the left mouse button, drag the cursor to the right to highlight the baseline range and set the post-transition baseline to be considered, and then release the mouse button. The slider controls below the graph can be used to visually zoom into a region of interest on the temperature axis. If desired, the upper and lower temperature limits of the data to be analyzed can be adjusted by dragging the end of the gray bar

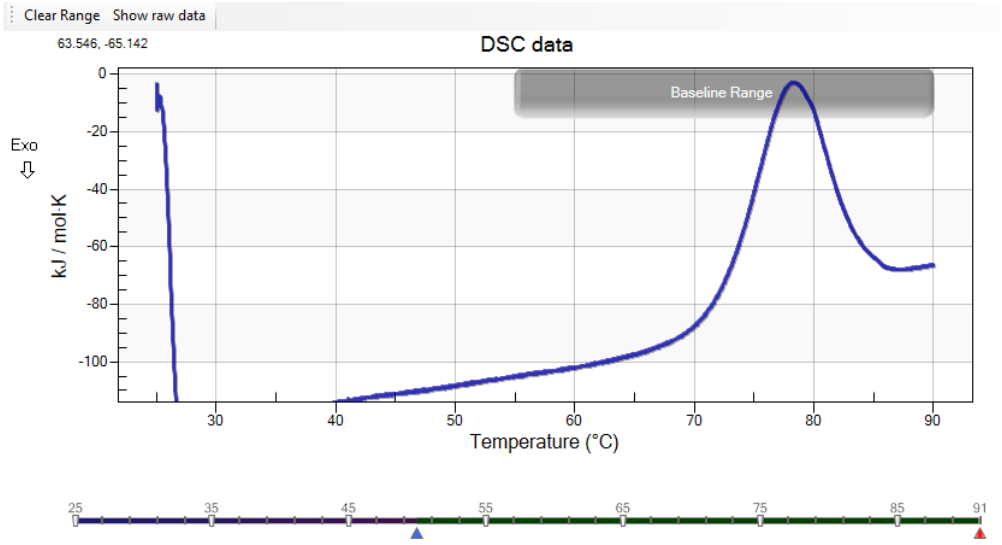


Figure 38 Highlight the baseline range.

2 Select the **Integration Baseline** tab.

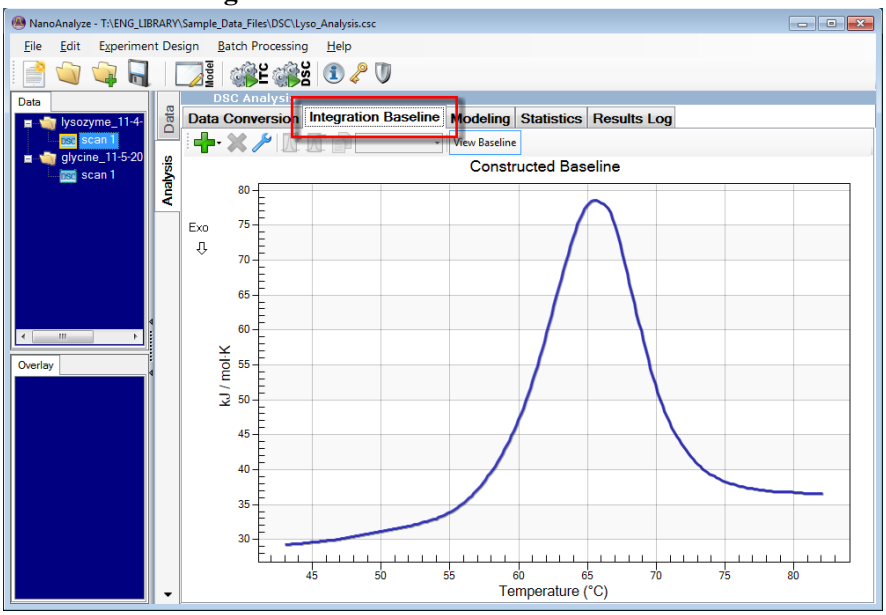


Figure 39 Integration Baseline tab.

3 The following functions are located on the **Integration Baseline** toolbar:

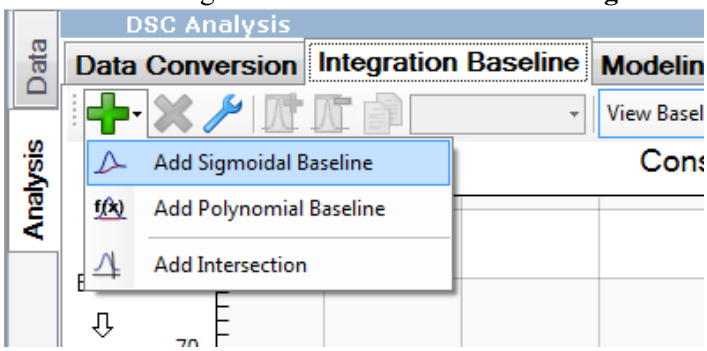


Figure 40 Integration Baseline toolbar.

- **Add Baseline or Intersection object**
- **Delete the selected baseline object…**
- **Settings**

4 Set the pre- and post-transitional baseline by moving the cursor into the graph display area. The cursor becomes a plus **(+)**. Place the + on the left of the peak where you want to start the pre-transition baseline, hold down the left mouse button, drag the cursor to the right to highlight the baseline range and set the post-transition baseline to be considered, and then release the mouse button.

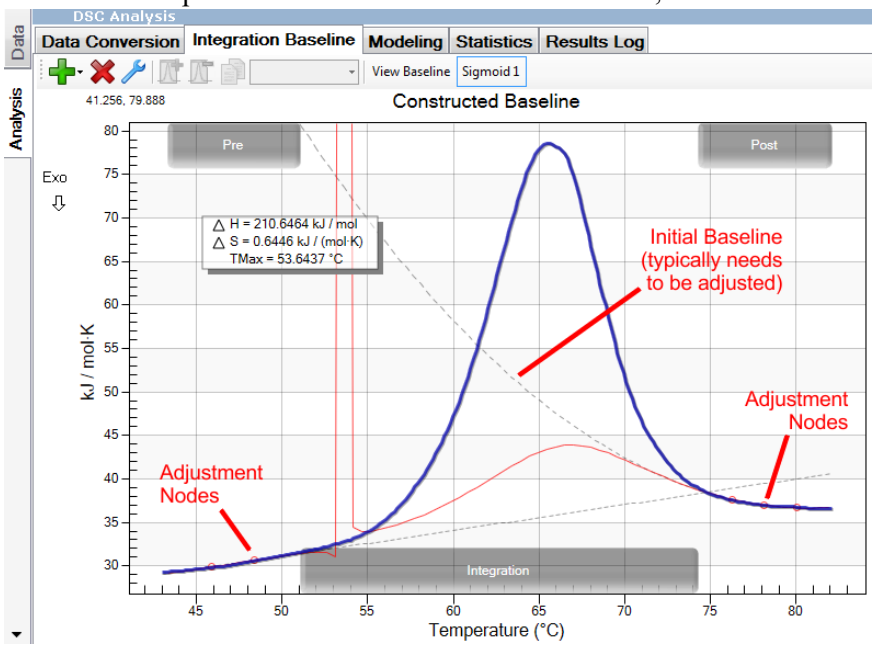


Figure 41 Set the pre- and post-transitional baseline.

5 When the mouse button is released, the preliminary baselines are fitted. The dashed lines are the Pre and Post baselines, the red solid line is the overall baseline with a smooth transition, and the gray circles represent “handles” that can be used to adjust the baseline slopes and curvature. There are two handles for a linear fit; higher order models will have additional handles.

6 Adjust the baseline fits by moving the handles within the chart. In the example in Figure 42, the second-order baseline on the right was adjusted to remove the curvature. Note that the handle can be moved out beyond the actual post baseline region, for ease of accurate adjustment. The enthalpy is calculated on a molar basis at this point.

7 Manually adjust the pre- and post-transitional baseline by clicking and dragging the handles to give a sigmoid curve baseline.

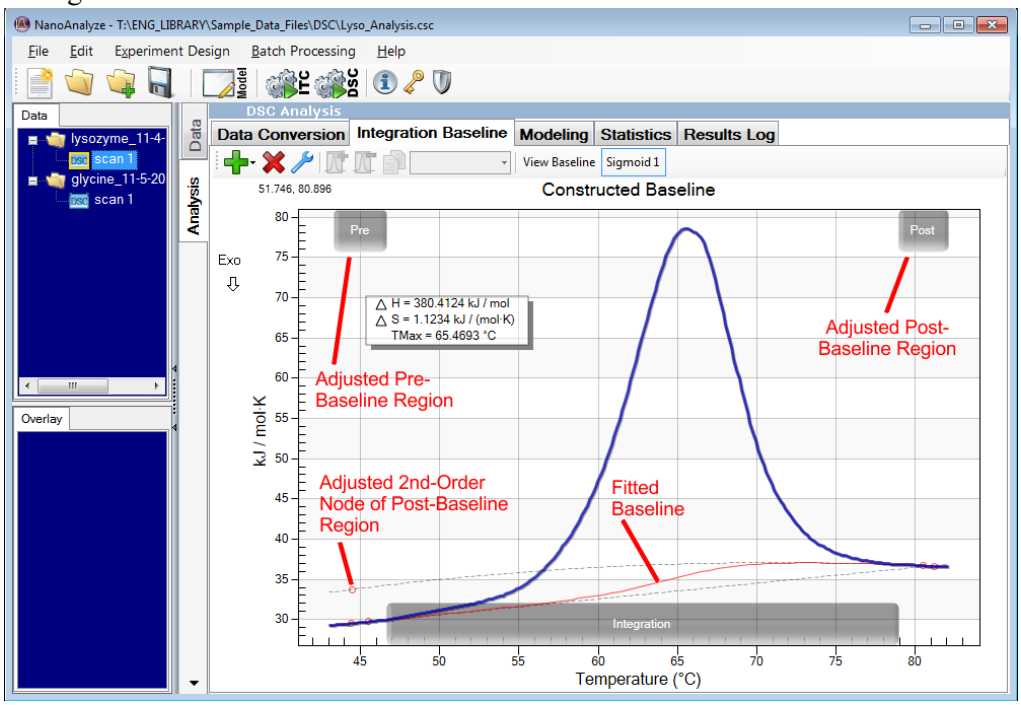


Figure 42 Manually adjust pre- and post-transitional baseline.

Fitting a Model

1 Select the **Modeling** tab. The following toolbar functions are available:

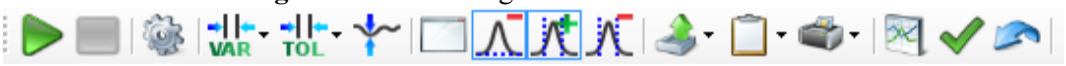


Figure 43 Modeling toolbar.

- **Start Fit**: Starts the model fitting process
- **Stop Model Fit**: Stops the model fitting process
- **Fit Settings**: Set/Adjust the model fitting parameters (Max fit time (s); Precision; Maximum iterations)
- **Variable Constraint**: Adjusts the permissible size of changes of parameter values that are allowed between fitting cycles
- **Fit Tolerance**: adjusts how close the fit must be before declaring the fitting to be complete
- **Stop at First Local Minimum**: If desired, activate the button so that during the fitting, when the first local minimum is reached, iterations will stop on the first occasion that a minimum of the fit

residuals is found. When activated, there will be a blue frame around the button. If not activated, the fitting continues until other exit criteria are met

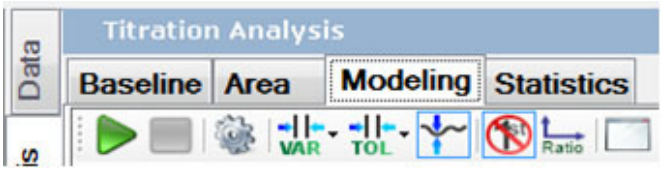


Figure 44 Activated **Stop at Next Local Minimum** control.

- **Full Screen Display**
- **Subtract Baseline**
- **Add T_m Region to be Used in the Fit**
- **Delete T_m Region**
- **Export to File**
- **Export to Clipboard**
- **Print**
- **Create Overlay Graph**
- **Save Current Settings as the Graph Default**
- **Reset Graph Settings to Defaults**
- **Move Results Box:** The thermodynamic results box can be moved to any convenient position in the graph by clicking and dragging with the mouse cursor. The font size may be changed in the Graph Settings pop-out menu at the right side of the window.

2 Click the asterisk * under **Model** to select the appropriate fit model.

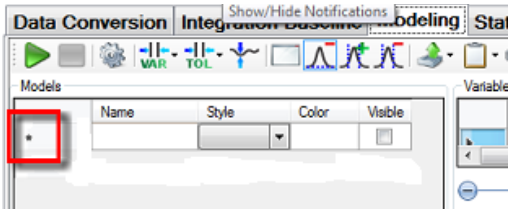


Figure 45 Click the asterisk (*).

3 Click the control labeled **Model** to bring up a dialogue box that lists the available models that are stored in the program. Click the + to show the list of available models.

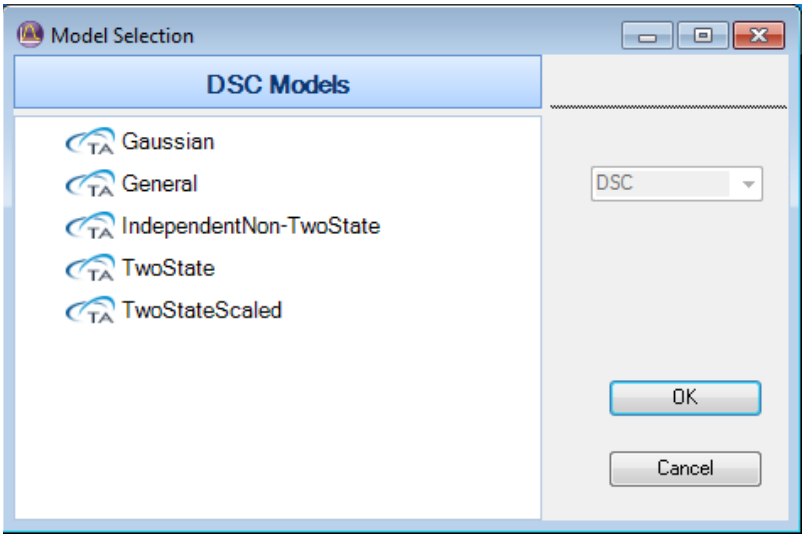


Figure 46 Model Manager.

4 Highlight the desired fitting model, then click **Select** to proceed. Multiple models can be selected together by holding down the Ctrl key while clicking on the second selection.

5 Available models are:

- **Two State**: Typically used for single transition peaks
- **Two State Scaled**: Typically used for single transition peaks and particularly useful in adjusting protein concentrations in the cell
- **General**: Typically used for single transition peaks when calculation for heat capacity is required
- **Gaussian**: May be useful when analyzing multiple overlapping transitions.
- **Independent Non-Twostate**: Use this model to analyze systems that unfold in the following path: **Native** > **Intermediate** > **Unfolded**.

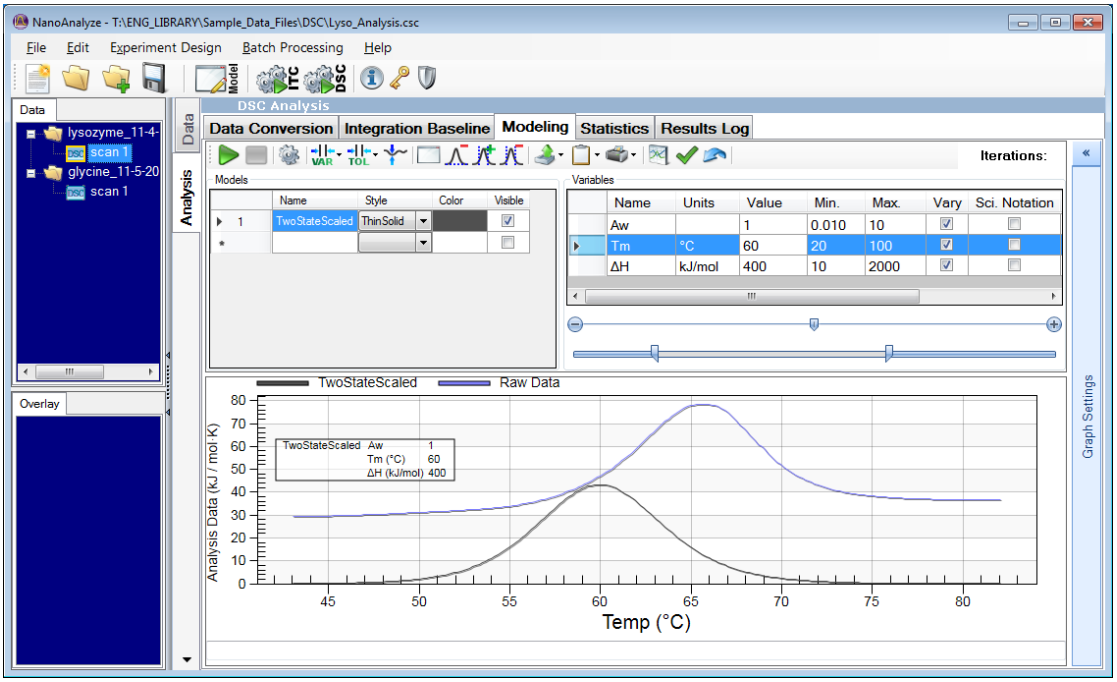


Figure 47 Models.

Multiple models may be selected in this window, and multiple instances of a model may be included. A typical use for this is to model multiple transitions. Click **OK** when you have selected the desired model or models.

Hint: In some cases, having too many degrees of freedom can slow down the fitting process. When this happens several things can help:

- Use the slider controls to obtain an approximate initial fit
- Temporarily disable some variables from the fitting process
- Adjust the limits of the acceptable ranges of the variables

Be sure to run a final automated fit with all parameters enabled. In particular, it is often helpful to impose strict limits on the available range of T_m for each model.

6 Place a check mark in the **Subtract Baseline** box.

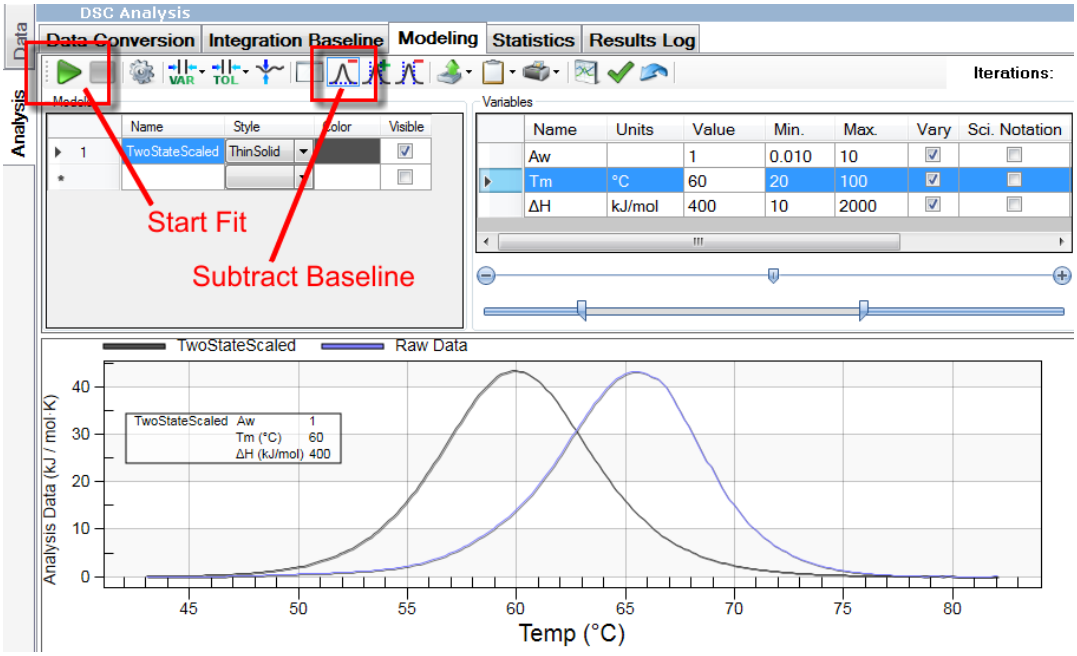


Figure 48 Select Subtract Baseline.

7 Click **Start Fit**. The red line represents the fitted model, and the calculated thermodynamic data updates in the **Variables** section of the screen.

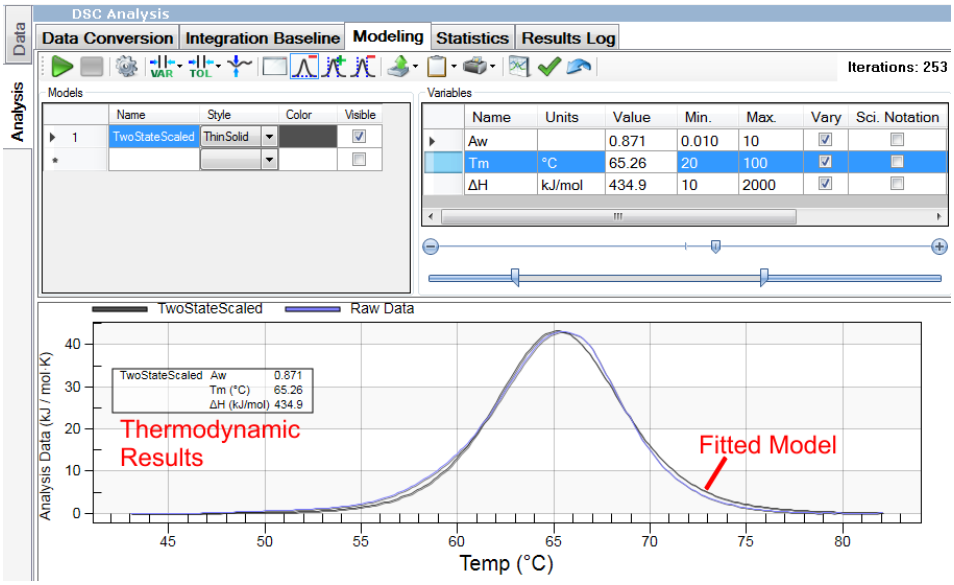


Figure 49 Thermodynamic data.

8 The final calculated data displays in the fit parameters box at the top of the data analysis window and in the data box inside the graph. To adjust any starting numbers, go back to the **MHC Conversion** and **Integration Baseline** tabs and make desired changes, then refit with the model.

9 The **Residual Plot** displays at the bottom of the fitted graph. This displays how close or far away the fitted curve is from the actual raw data.

DSC Batch Processing Mode

NanoAnalyze can rapidly perform simple analyses on groups of DSC data files with the Batch Processing feature. Basic thermodynamic values are calculated and MHC conversion is available. Batch processing provides a rapid preview of the basic DSC experimental results Tmax, ΔH and ΔS. Experiments that warrant further, more detailed attention can subsequently be modeled in the Modeling tab of NanoAnalyze. All processing parameters and initial thermodynamic results that result from batch processing are passed back to the individual data files for convenience.

1 First, load a collection of files into NanoAnalyze. Multiple files can be selected as a group by using the standard controls within the **File** > **Open** or **File** > **Add Files** dialog boxes. To open a group of files, click the first file to select it, then use Shift-Click on the final file in the set (hold the Shift key while clicking the mouse button). All the files in the range will be highlighted. Alternately, individual files can be added to the multiple selection by using Ctrl-Click for each file (hold down the Ctrl key while clicking the mouse button). Then click **Open**. Check the file tree on the left side of NanoAnalyze to verify that the desired set of files for batch processing has been loaded.

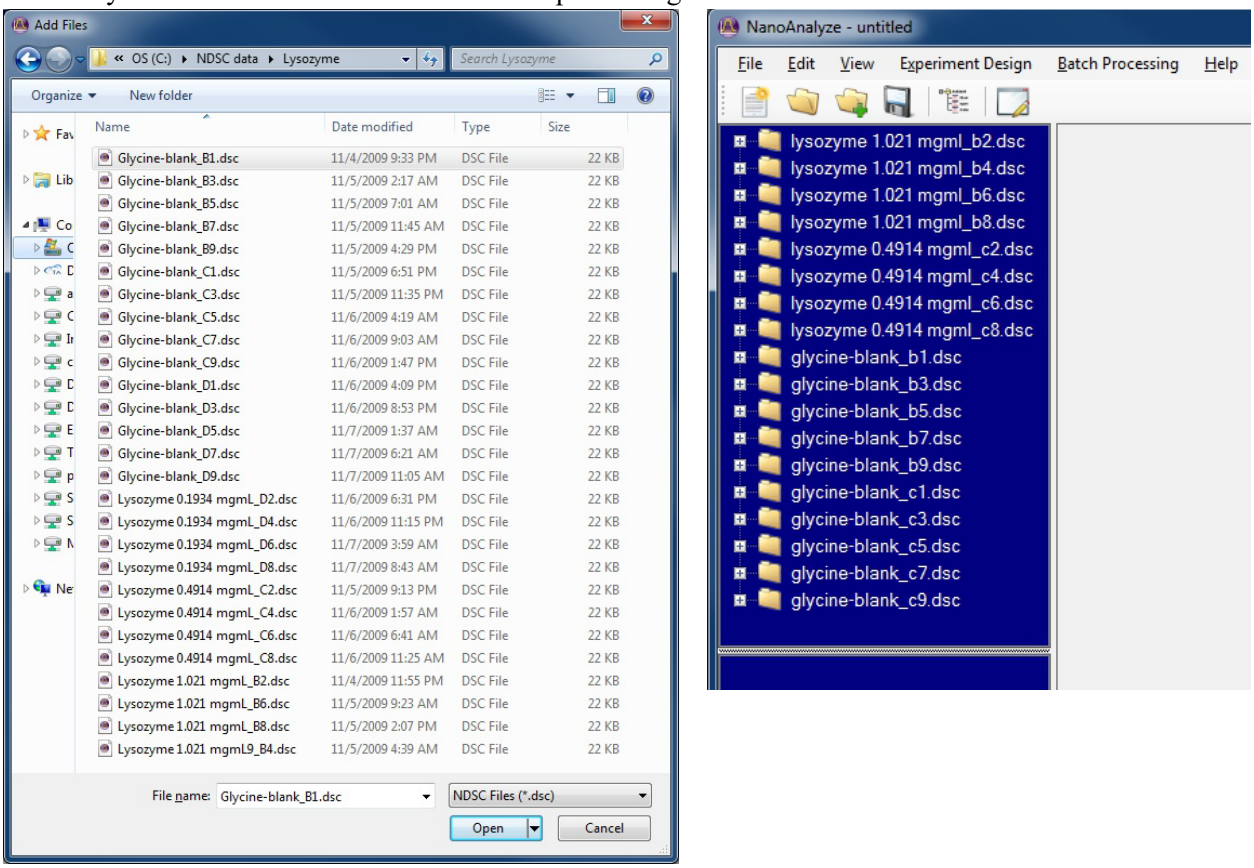


Figure 50 Load files (left figure), file tree (right figure).

2 Select **Batch Processing**>**DSC** from the menu bar.

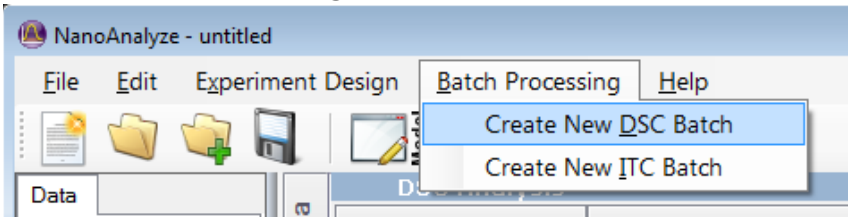


Figure 51 Entering Batch Processing mode.

The Batch Processing dialog box displays. The window is divided into three sections:

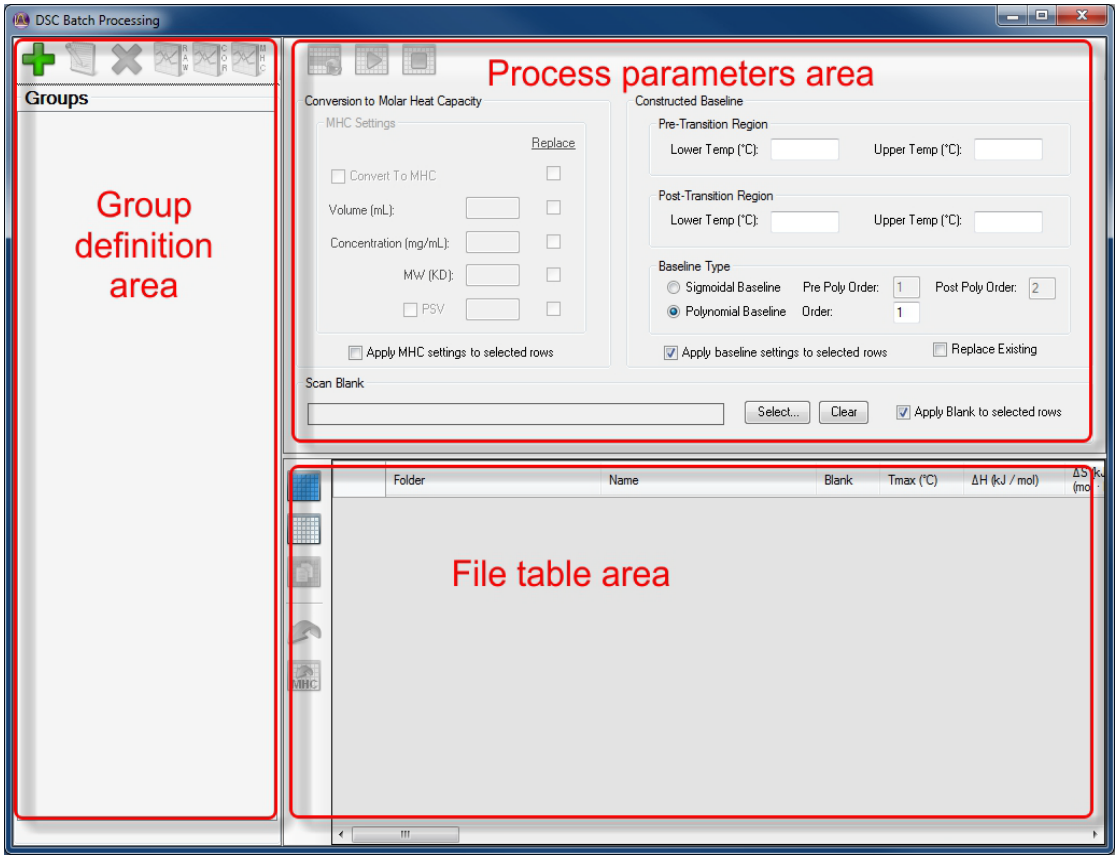


Figure 52 Batch Processing dialog box.

- The group definition area contains tools for assembling and previewing groups of files. Groups are displayed in the space below the Groups toolbar after the groups have been defined. The group is a tool to collect files which are to be processed in a similar way, completing the processing with a single click without duplicated effort. It is easy to set common parameters for the group but individual files can be assigned any unique settings as required. The results can be graphically previewed together as a set, and Overlay Graphs can be made for each group.
- The process parameters area is the data entry control to set the processing parameters for the selected file or sets of multiple files.

- The file table area displays the values of the process parameters along with the thermodynamic results from the processing. The scroll bar allows viewing of every column in the table. For convenience the file name always remains visible.

Add group

Edit group

Delete group

Create raw data overlay graph

Create corrected data overlay graph

Create corrected data overlay graph

Figure 53 Group icons

3 To assemble the groups, click the green + icon at the upper-left of the DSC Batch Processing dialog. The **DSC Group Items** dialog displays.

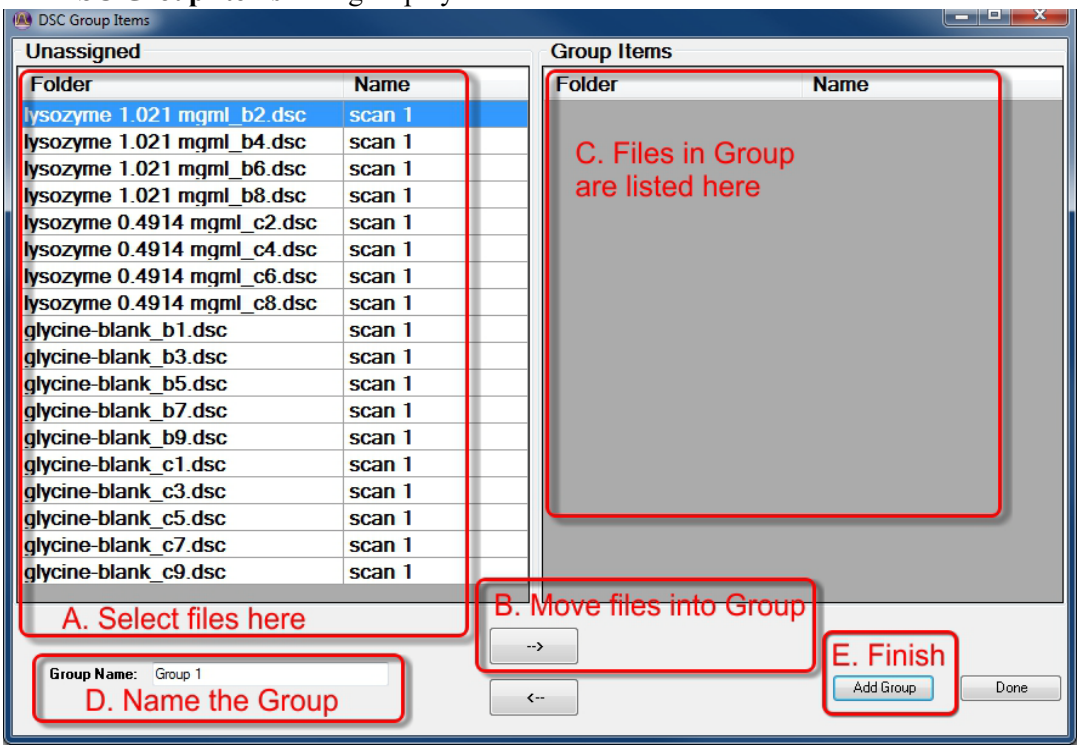


Figure 54 Group Definition dialog.

A Select the set of files that logically belongs together as a Group. The standard multiple-selection controls Shift-click and Ctrl-click are available.

B Click the right arrow button to add the selected files to the group. The labels for these files will moved from the **Unassigned** window (left side) over to the **Group** items window (right side).

C Files that have been assigned into the group will appear on the right side. If any files were accidentally added that do not belong to the group, they can be deleted by first selecting them on the **Group** side, then clicking the left arrow. The file labels will be removed from the **Group** window and will reappear in the **Unassigned** window.

Figure 55 shows a group that is partially defined and is ready to add more files.

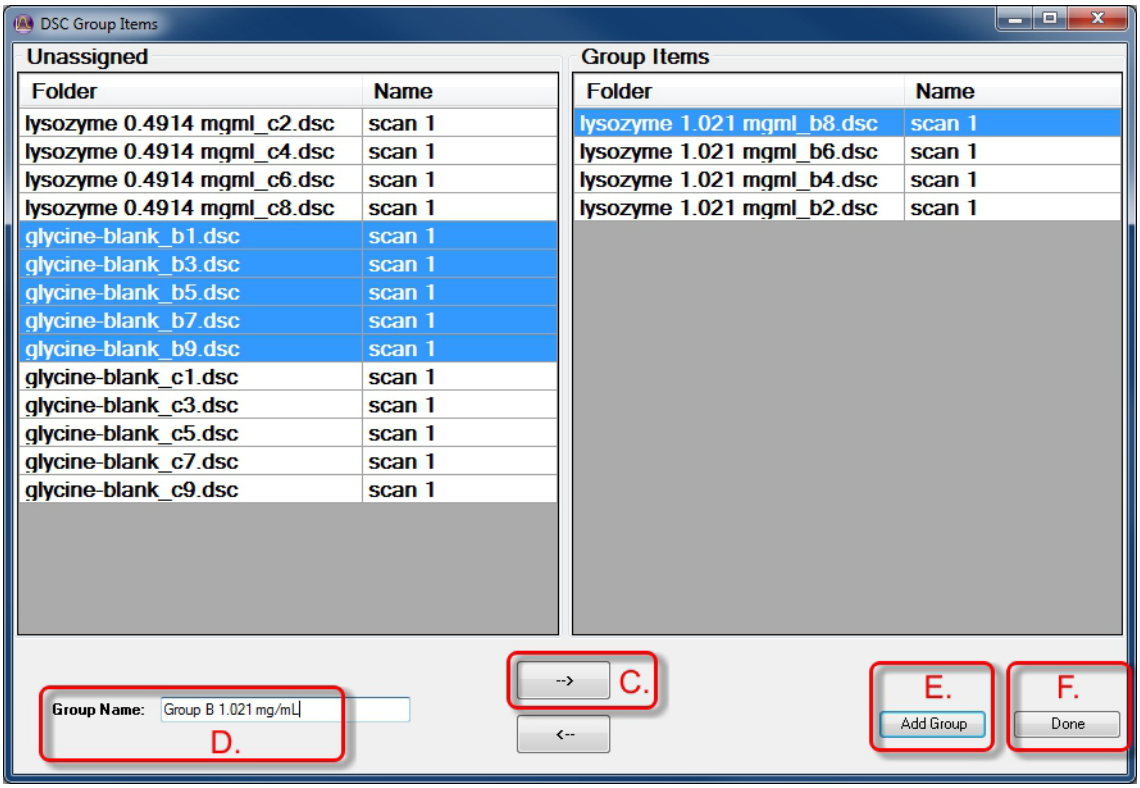


Figure 55 Defining a Group.

D If multiple **Groups** are to be created, provide a unique name for each in the entry box at the lower left (shown in Figure 55).

E When the list of files on the right side is complete and correct, complete the creation of the group by clicking **Add Group** (shown in Figure 55).

F If desired, more Groups may be defined by following the above steps A through E again. When all desired Groups are created, click **Done** on the lower-right portion of the screen.

When the desired groups have been created, click **Done** to return to the Batch Processing window. Figure 56 shows the layout of the window and the sections of related data file table editing controls.

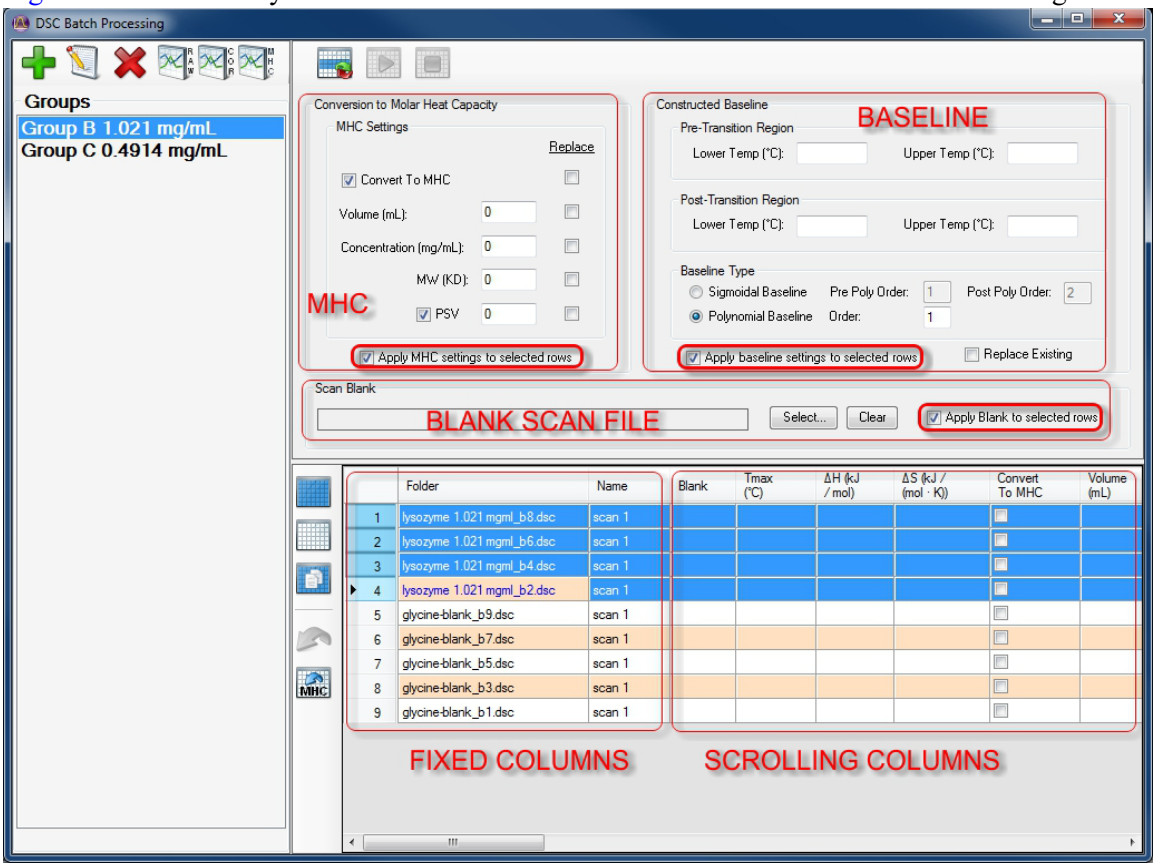


Figure 56 Batch processing controls.

4 Next, define the process parameters. The processing variables can be set up the same for all members of a Group, or with individualized settings for any member as needed. The first step is to select the lines in the table that are to be edited.

Several of the icons at the left side of the File Table Area provide functions that assist with the file selections.

Select all rows in table

Deselect all rows in table

Copy selected rows in table to Clipboard

Figure 57 Table functions.

Mouse clicks can be used along with the Shift or Ctrl keys as appropriate to assist with making the selection. Figure 56 shows four data file rows selected, as indicated by the blue highlighting.

When the desired rows have been selected, changes or additions to the process settings may be entered at the top of the page in the Process Parameters area. Sections are provided for parameters associated with blank experiment scans, molar heat capacity, and baseline. Select the **Apply** checkbox for any section where changes to settings will be made. When all the desired settings have been entered, click on the **Update table with settings** icon  .

To ensure that changes to settings are updated in the file table, check to see that all enabling settings such as the appropriate **Replace**, **Replace Existing**, or **Apply** boxes are checked.

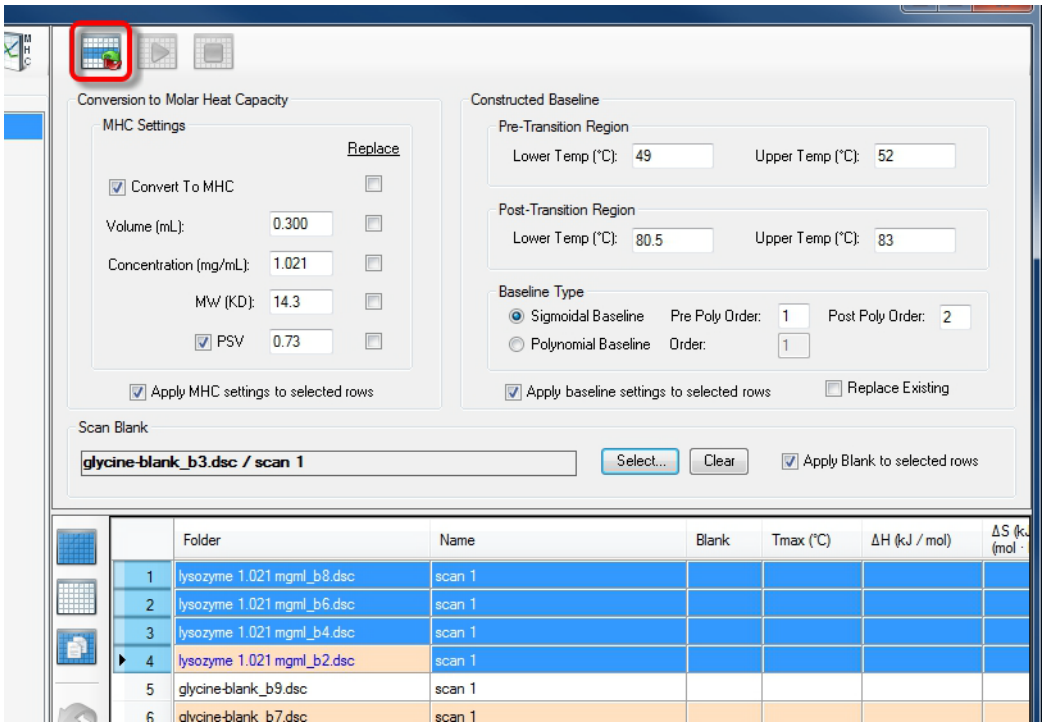


Figure 58 Entering changes to process settings.

After entering new settings into the table, every cell with altered settings will appear with a red highlight, as seen in the **Blank** column in Figure 59. Cells which are altered but not selected will appear with a yellow highlight.

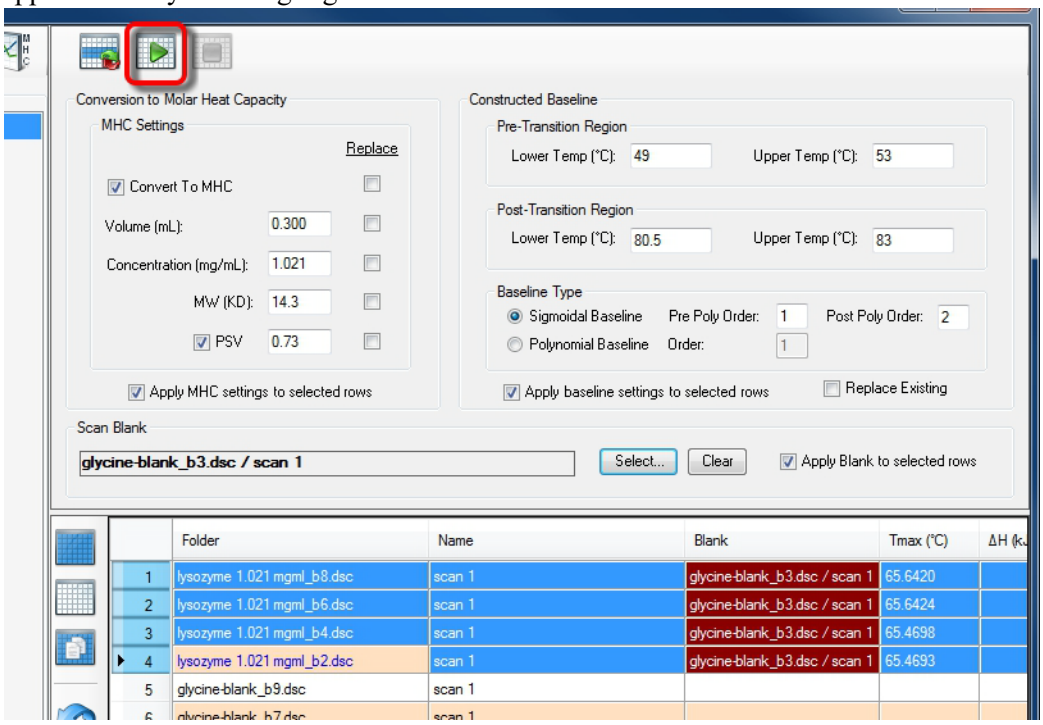


Figure 59 Files ready to process.

5 The next step is to process the data files. The button for the action **Apply table changes to the dataset**  will now be enabled; click the button to process the datasets.

Each selected data file row will be processed one by one in sequence. For each line, blank data files will be subtracted, baselines will be calculated according to the "pre" and "post" ranges, the baselines will be subtracted, and thermodynamic variables will then be calculated. The thermodynamic results will be filled into the table as seen in Figure 11.

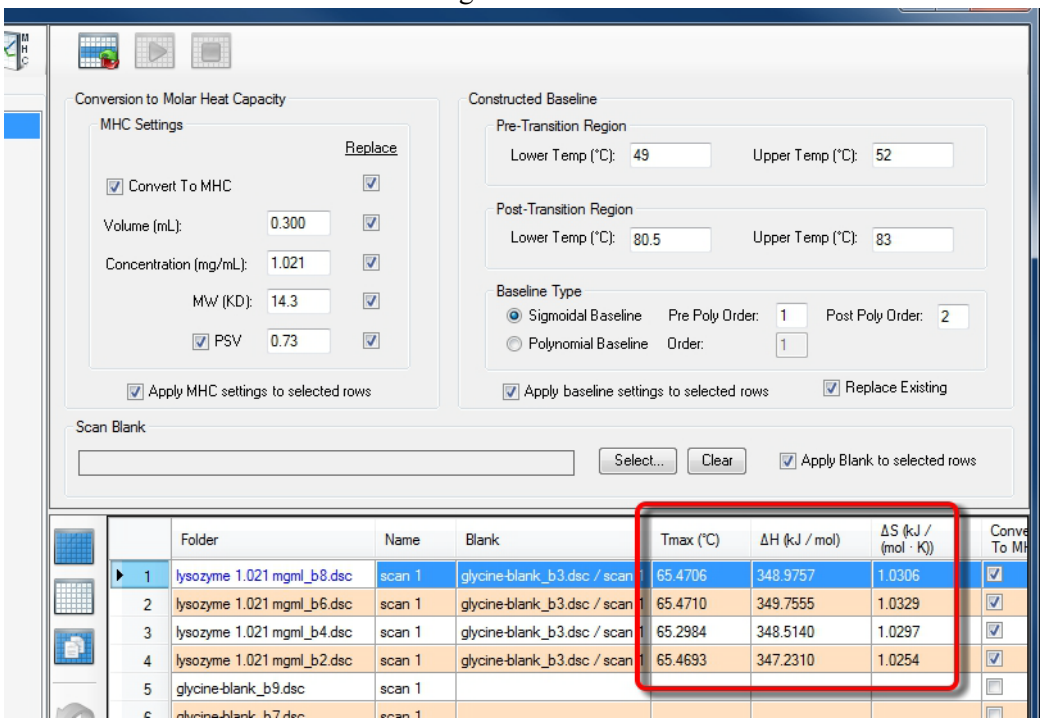


Figure 60 File processing completed.

If unexpected results arise, this may have been caused by signal noise causing changes in the details of the baseline paths. Exit batch processing mode, select the file(s) in question, and view the **Integration Baseline** window. The baseline can be shifted by moving the adjustment handles (the small open circles). When the baseline is redrawn, the thermodynamic values will be updated. If any files warrant further study, you can proceed to the **Modeling** tab.

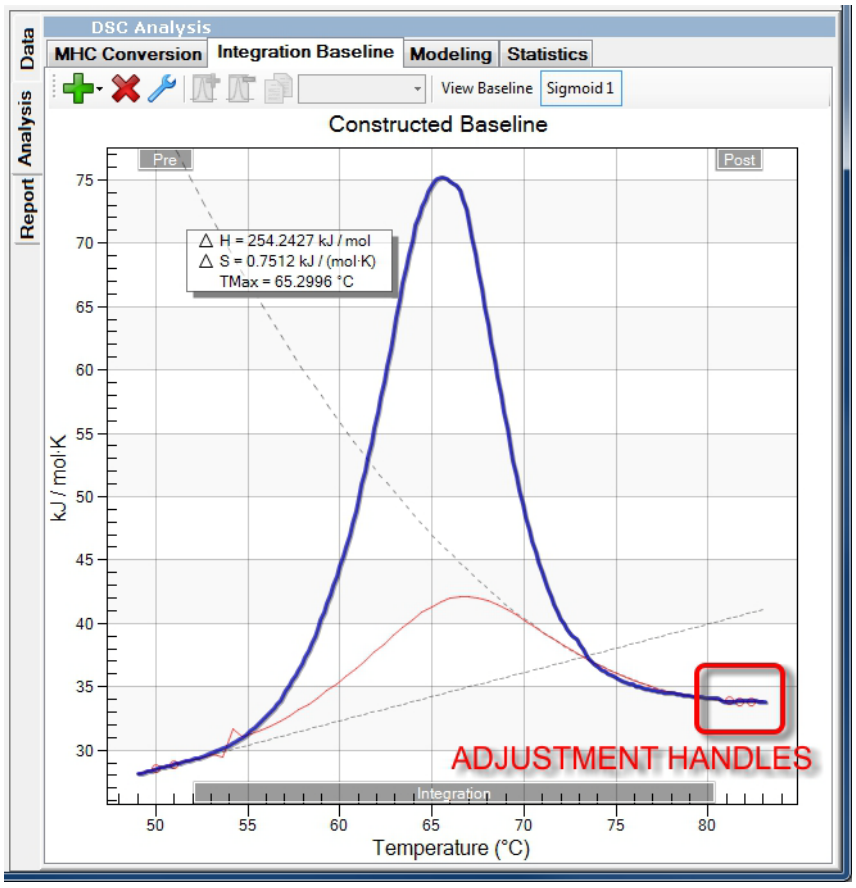


Figure 61 Example of a problematic baseline, in this case needing a manual adjustment.

NOTE: All data is fully synchronized between the batch processing and the standard modes of using NanoAnalyze. Any change to a data file setting made in either mode will also be present in the other mode. You can save the work on the full set of files by saving in the .CSC format from the **File** menu in the NanoAnalyze main window.

ITC Batch Processing Mode

Start by loading several data files into NanoAnalyze. For convenience they can be organized into a common folder. Click on the blue **Data** area and right-click the mouse, then select **New Folder.** The folder can be renamed if desired.

Optionally, an Overlay Graph may be created at this point to review the files before processing. Create an overlay Graph by right-clicking in the blue area in the **Overlay** group and select **New Overlay Graph**. Drag and drop the file folder from the **Data** group into the area labeled **Datasets to Graph / Drop primary graph items here**.

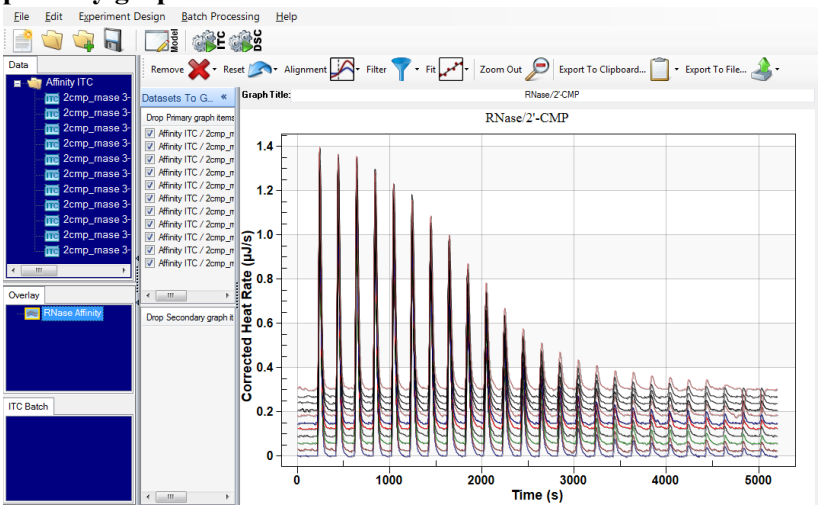


Figure 62

Create a new ITC batch by clicking on the icon. This opens a dialog box for selecting which data files are to be included in the batch.

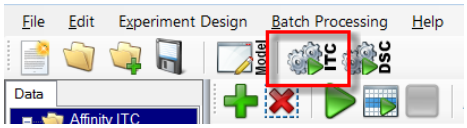


Figure 63 Batch Processing > Create New ITC Batch

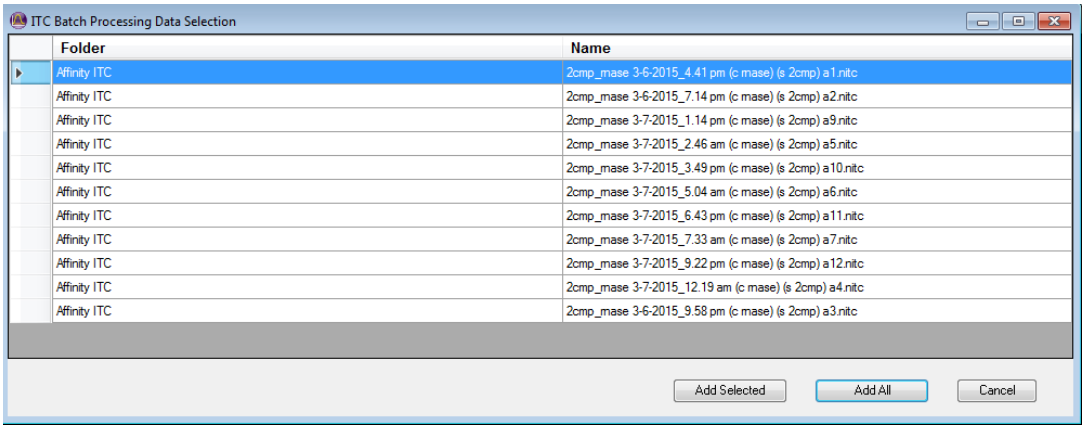


Figure 64 ITC Batch Processing Data Selection.

A new main window for ITC batch processing displays.

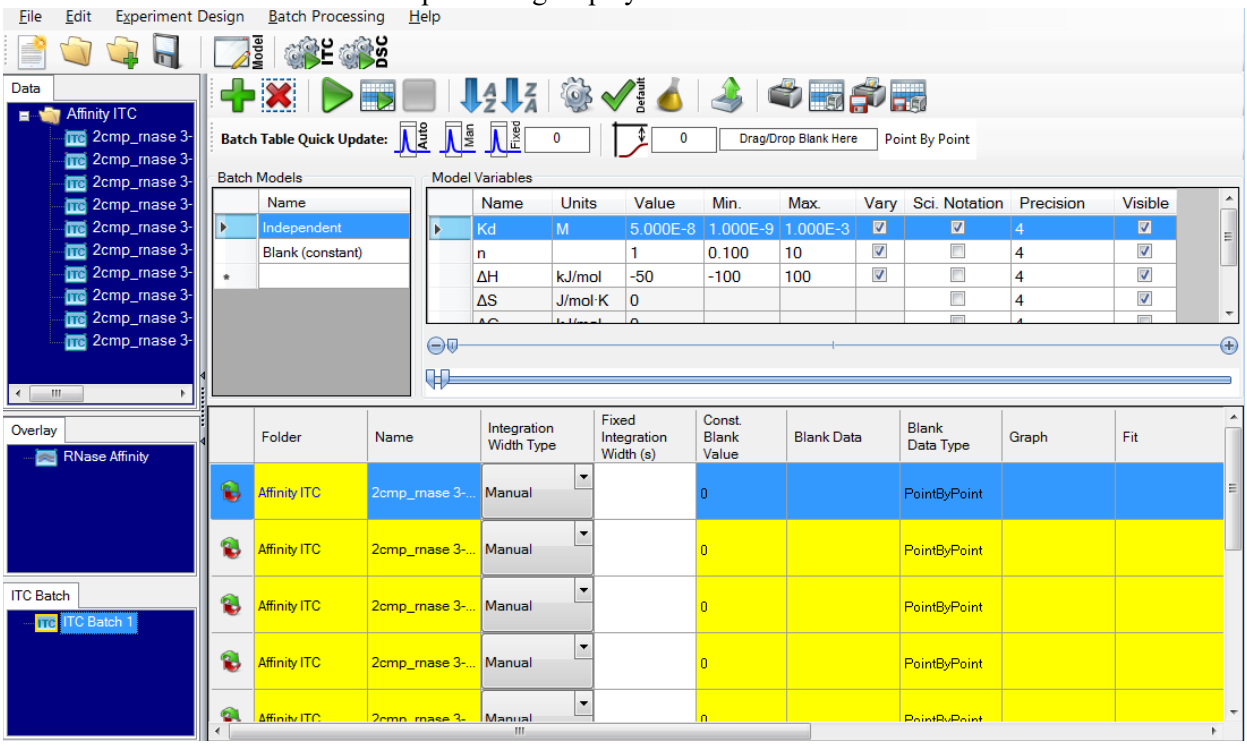


Figure 65 ITC Batch Processing window.

Tool bar item descriptions:

Figure 66 Toolbar.

Add and **Delete** files

Calculate all files, **calculate selected files**, **stop calculation**.

Sort in ascending order, **sort in descending order**: The order is determined by the calculated thermodynamic values, each category multiplied by its assigned weighting value.

Settings: Launches the Settings dialog box.

The Settings dialog box allows the user to input a weighting function to be used as data sorting criteria. The default weighting factors are all 1.0. Use a higher weighting factor on a parameter to increase the

emphasis during the sorting. Use lower factors to decrease the emphasis. Only the relative values of numbers matter.

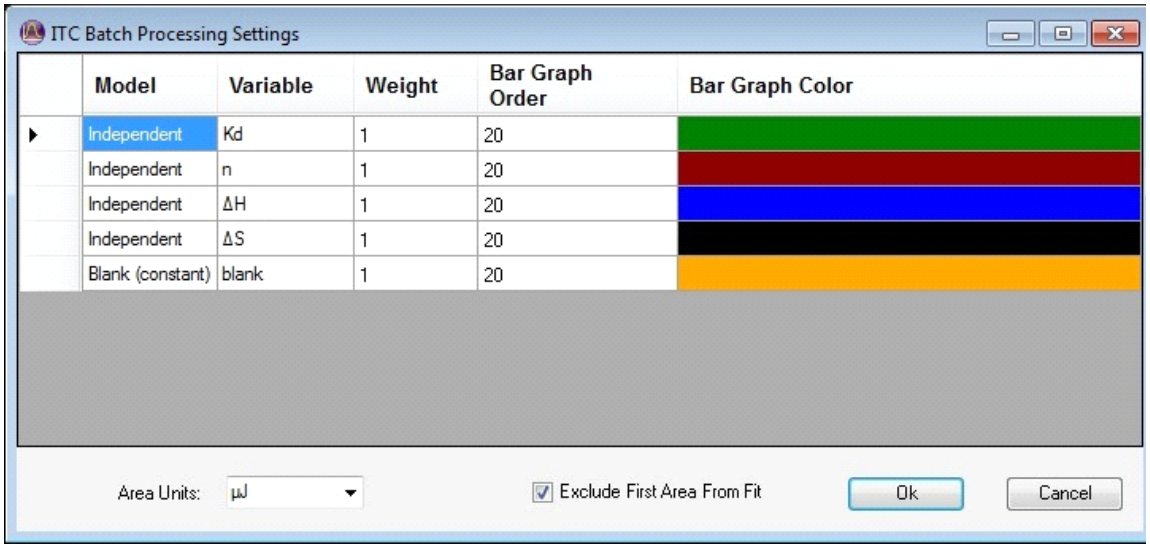


Figure 67 Settings dialog.

Set Defaults: Sets the current selection of models as the default choice when creating a new ITC Batch process.

Experiment Parameters: Launches the Experiment Parameters dialog box.

The Experiment Parameters dialog box provides access to adjusting experiment parameters such as the sample data and analysis temperature, and applying the changes to all loaded files, or to the selected files only.

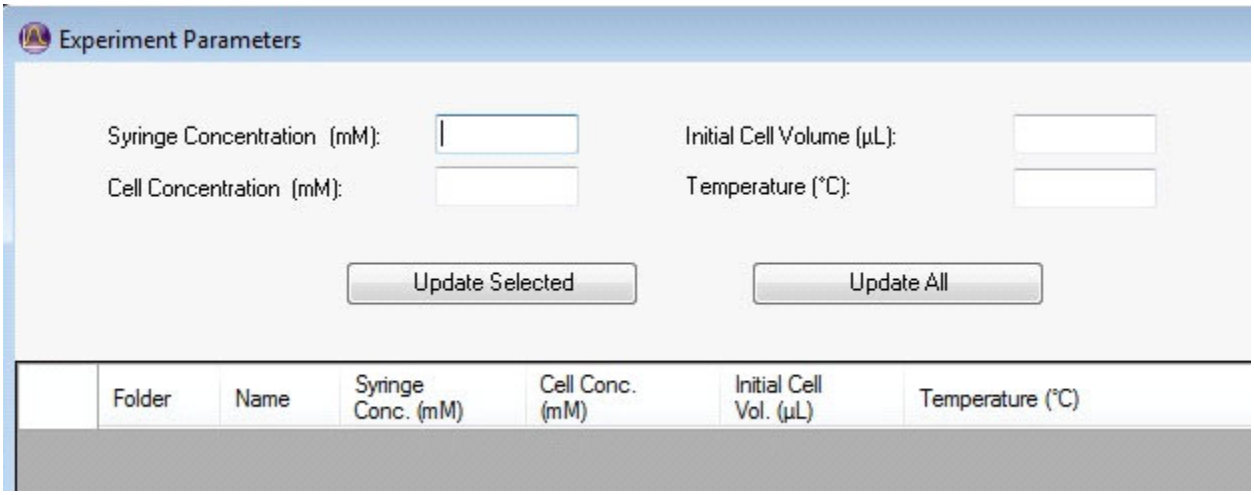
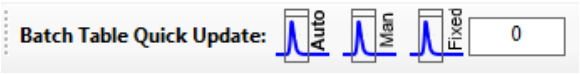


Figure 68 Experiment Parameters dialog.



Integration Window controls: The Auto button automatically fits baseline nodes and integration windows to the data based on automatic interpretation. This generally gives the best results. When the signal levels are very low it might become necessary to manually set the integration windows. Enter a time value in seconds for a different integration window width, select the files the change is to be applied to, and then click the graphic icon to apply the changes. Note that the default integration width is the same as the injection interval. Use this control to set a narrower window for integration. Use this only to exclude regions of stable baseline between injection peaks.

Injection Blanks controls:

- Update selection with new values
- Enter a constant to be used for all peaks (option 1)
- Drop a blank file to be used on a point by point basis (option 2)
- Point by Point/Average selection toggle button

The typical method for NanoAnalyze model fitting of ITC data is to include a Blank (Constant) model along with the binding model in order to automatically calculate blank values for the peaks in which little to no reactions occur. However this may not be practical if the full titration curve is not realized in the experiment. As an alternative, blank data can be obtained separately. A constant value can be entered, and then the graphic icon in this group would apply that blank value to all peaks. Another method is to run a Blank Experiment with the same timing as the Sample Experiment, then drag and drop the file into the provided window. In this case there will be an option to apply the blanks on a one-by one basis (peak 1 of the blanks is subtracted from peak 1 of the sample experiment, etc.), or the average of all the blank peaks can be used. Click the **Point By Point** icon to change it to "Average" or vice versa.

Export to File, **Print All Bar Graphs**, **Print Select Bar Graphs**: The export option outputs a table of all data into a plain text (.txt) file or a Microsoft Excel compatible file (.xls) for external processing or report generation.

Save all or selected images into .jpg files for printing

Adding files to be processed in a batch

Use the red "+" sign icon to open a dialog box in order to add files into the batch process selection. Make a multi-selection by using Ctrl-mouse click to add one file at a time, or Shift-click to select a group.

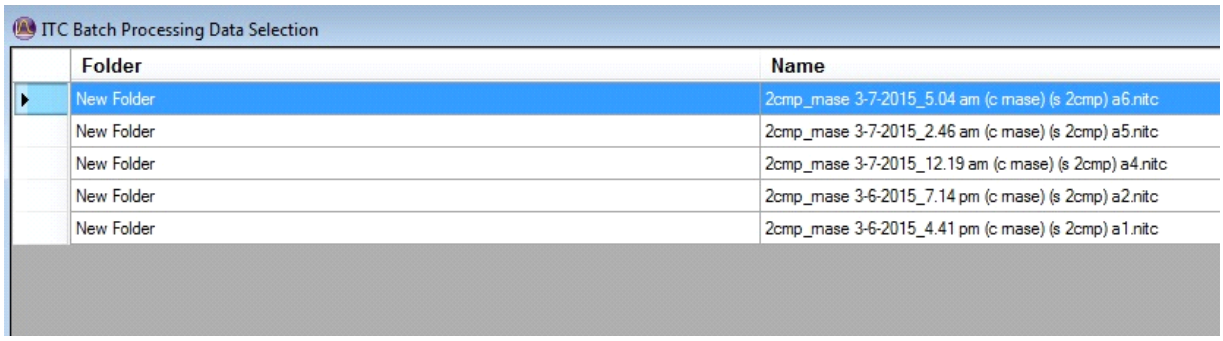


Figure 69 Add files.

Click **Add/Close** to complete the selection. The files will display in the batch data table at the bottom of the window:

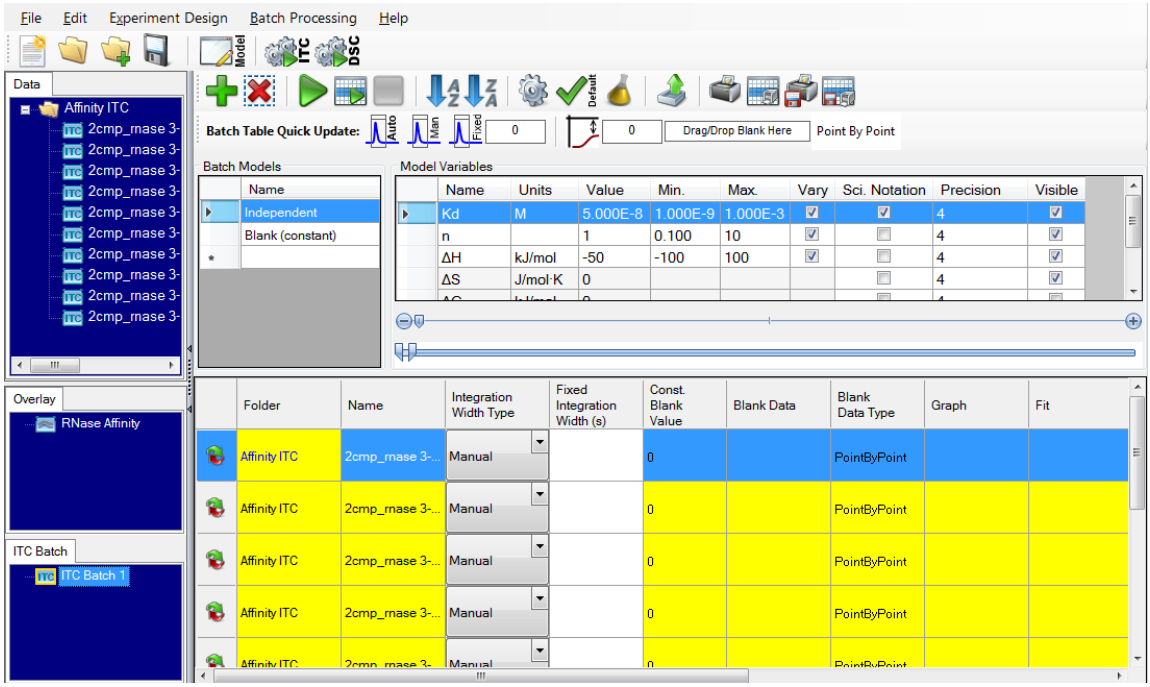


Figure 70 Files displayed in the batch data table.

Click on the "*" button in the **Models** area to bring up a dialog box for selecting models. Click **OK** when finished.

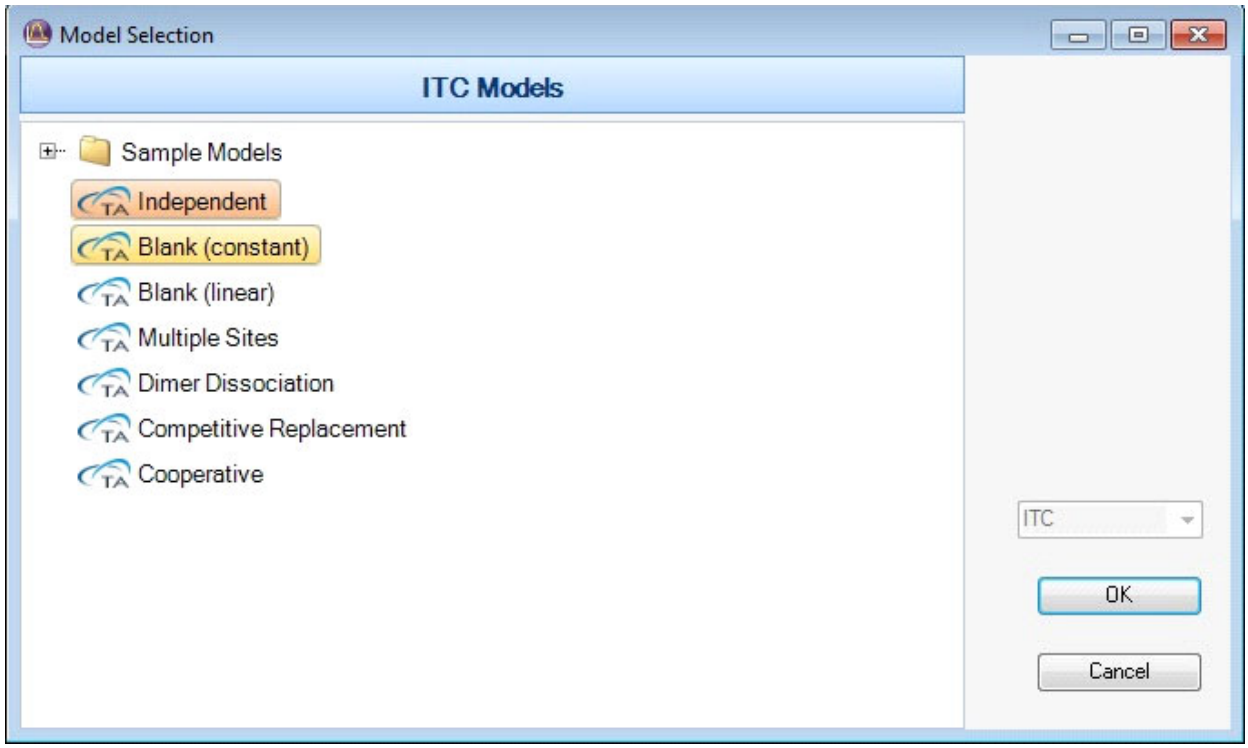


Figure 71 Model Selection dialog.

Click on the green arrow icon to start the model fitting. When completed, the thermodynamic data will display in the data table.

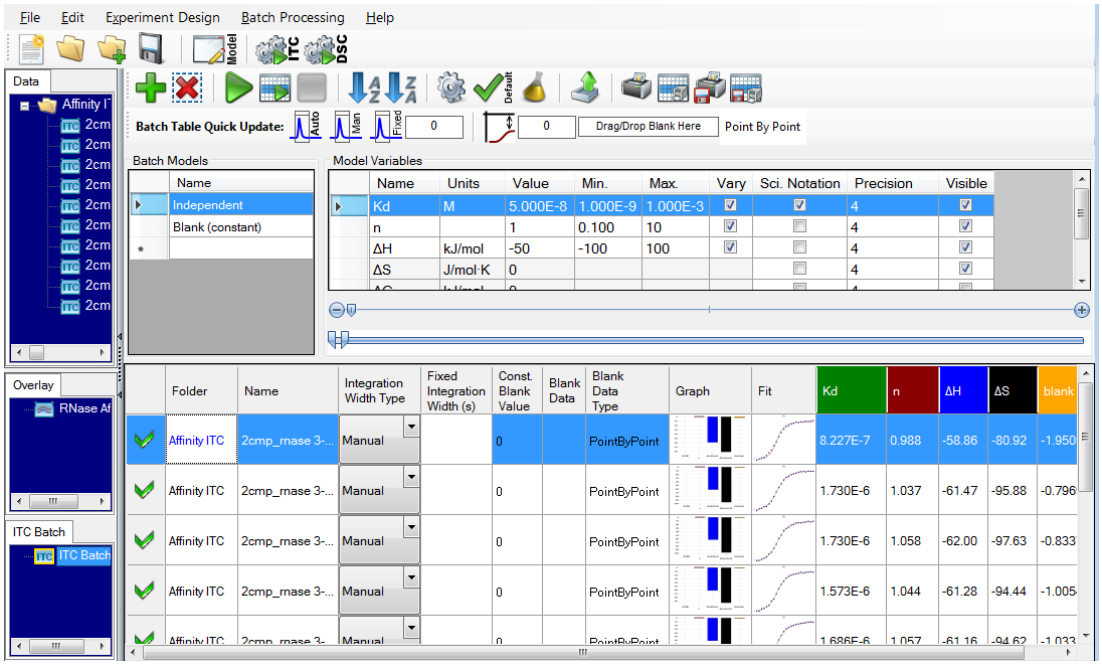


Figure 72

Hovering the mouse cursor over a data parameter column header will display a comparison graph of all files for that parameter.

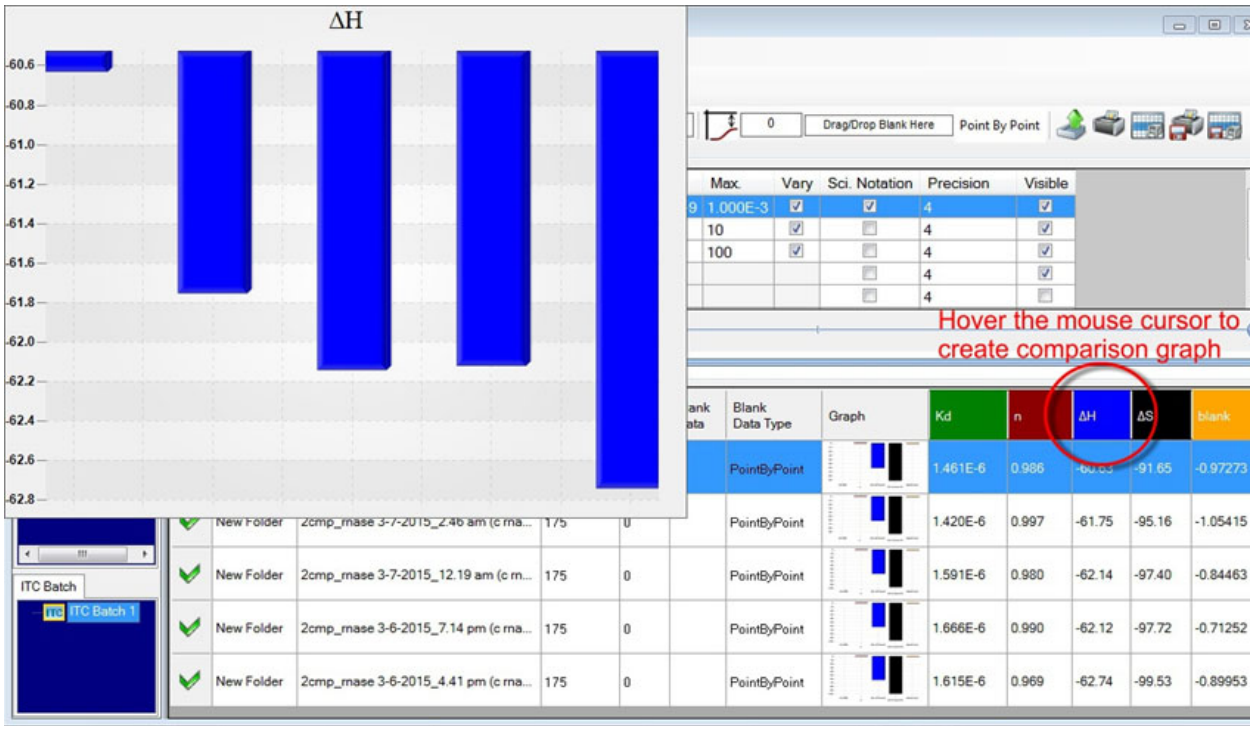


Figure 73 Comparison graph.

Add the fitted data to the Overlay Graph, if desired. Drag and drop the folder into the secondary graph area and the fitted curves will display.

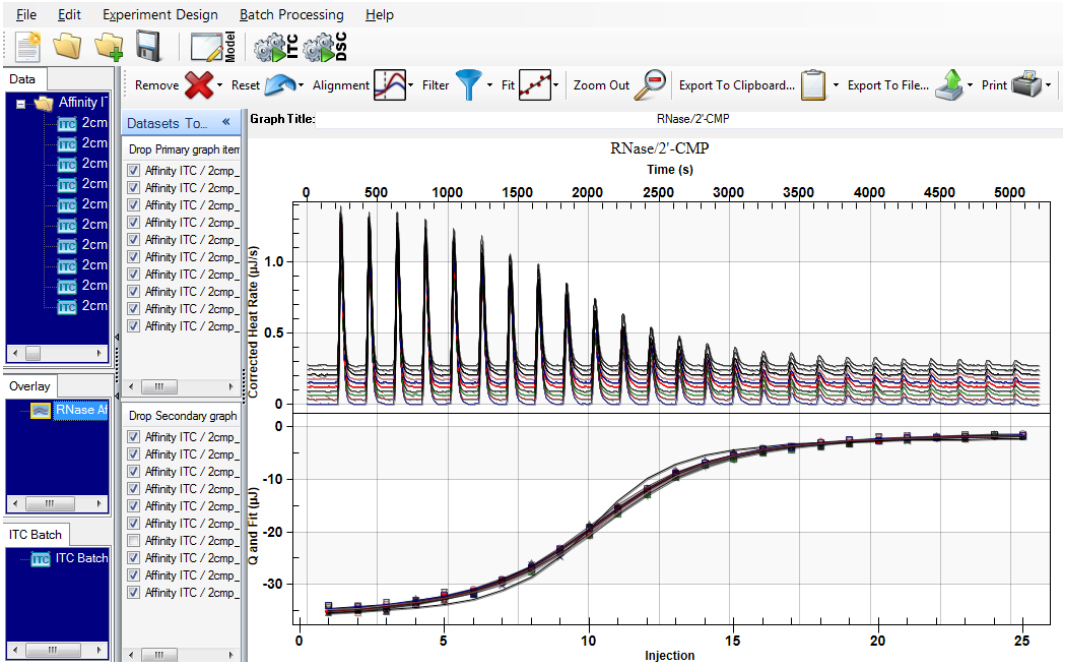


Figure 74 Fitted curves displayed.

Advanced Features

Statistical Analysis of Models

Once a model has been satisfactorily fitted to a set of experimental data the parameters are considered to be an optimal fit of the chosen model to the experimental data. This is represented with an example of an isothermal titration experiment, as shown below.

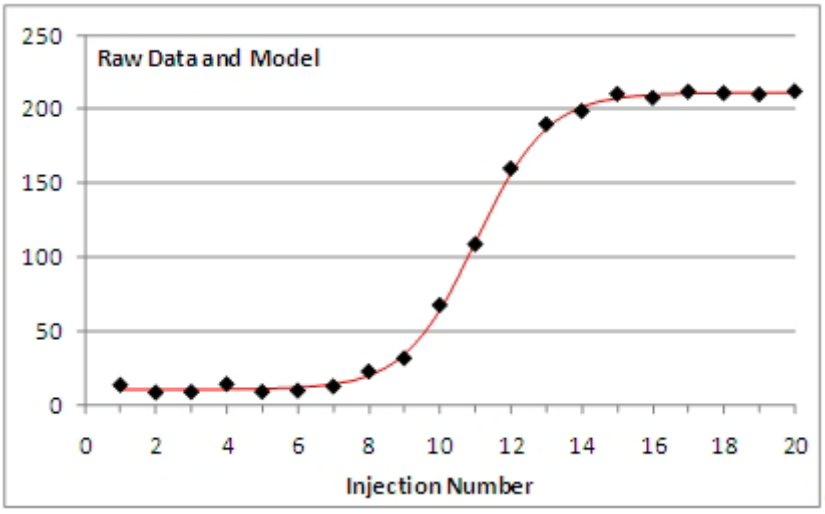


Figure 75 Isothermal titration experiment.

The **Statistics** page is used to calculate estimates of the uncertainty of the model parameters. In multiple trials, a defined amount of variation is introduced into the model and the fitting will be performed again. Each parameter will vary within a Gaussian distribution, which will be characterized.

The first step is to construct a reference set of "perfect" synthetic data points from the model curve. At the x-axis location of each experimental data point, the value of the model equation is calculated. These synthetic data points are idealized and therefore have no scatter, as seen below.

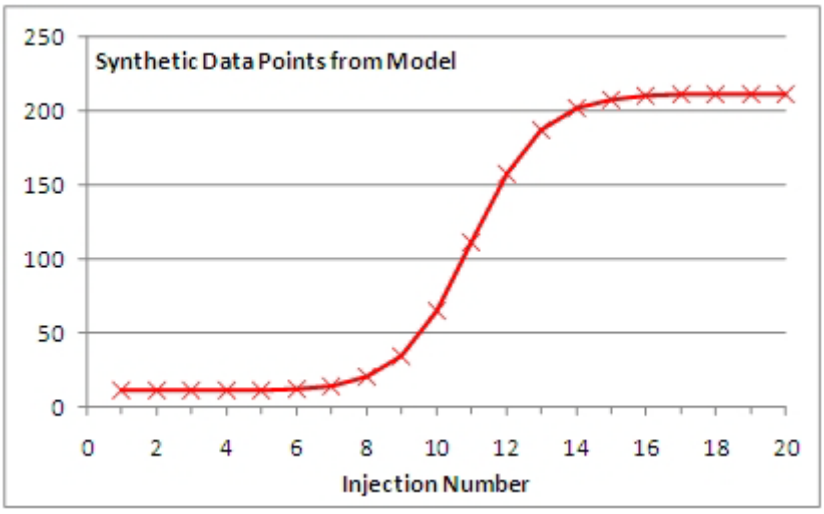


Figure 76 Idealized data points.

The next steps are to introduce a series of trials consisting of temporary perturbations to this idealized data set, re-fit the model for each trial, and record the resulting distributions of each model parameter (with the exception of the blank, if present). This repetitive process is represented below.

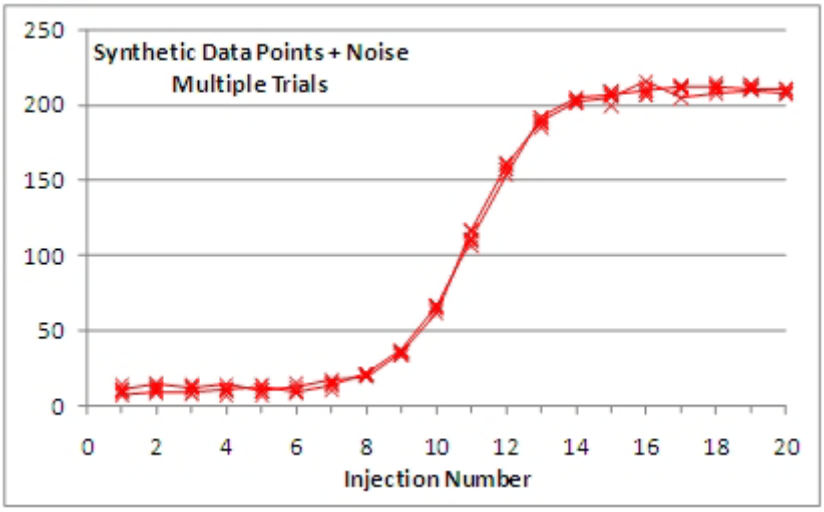


Figure 77 Multiple trials.

Random noise is superimposed onto the heat value at each of the X-axis data points in the reference set in a carefully controlled manner. Each change is randomly generated within a Gaussian distribution that has a standard deviation identical to that of the original data set. This figure is displayed on the **Modeling** page between the windows for the **Model** and the **Residuals**. Each model parameter will also tend to be normally distributed, provided that a sufficiently large number of trials have been performed.

Statistics Work Flow:

1 Fit a model (or multiple models) to the data from an experiment. Be sure that the fitting has been optimized, and that all changes to the model have been finalized before moving on to the **Statistics** page.

NanoAnalyze automatically transfers the values of the fit parameters and the standard deviation around the fit.

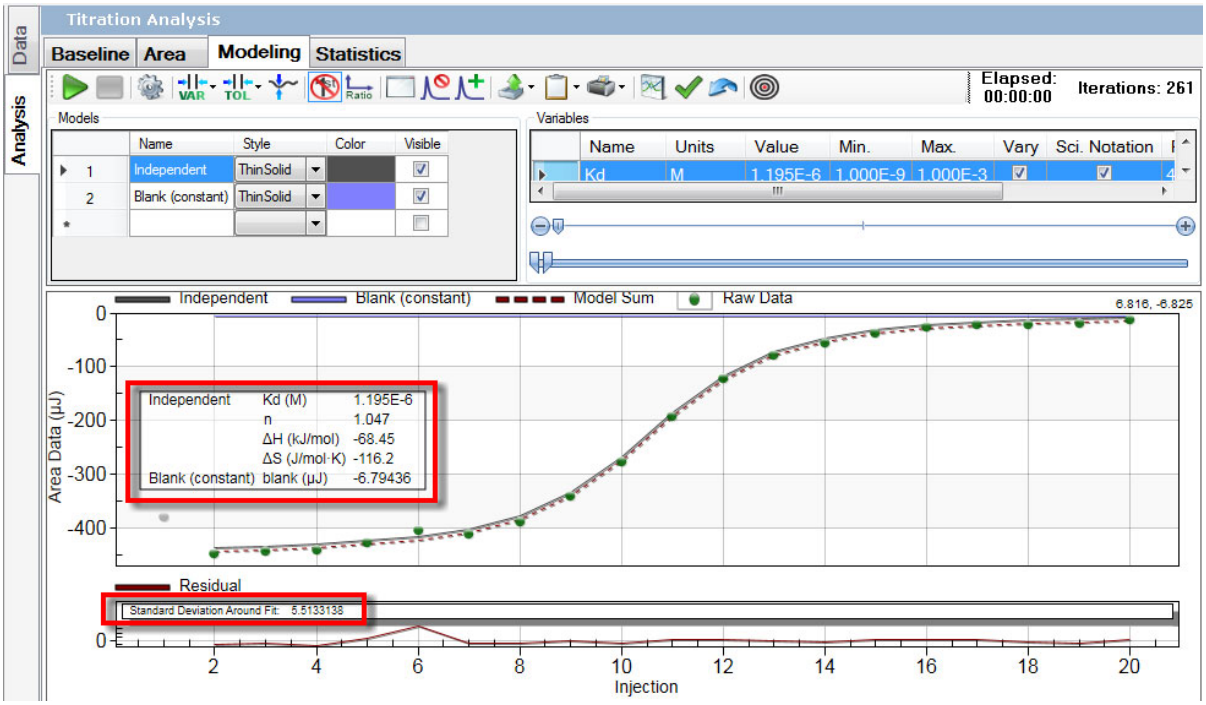


Figure 78

2 Proceed to the **Statistics** page.

NOTE: If a Blank model was used in the fit, uncheck the box next to the Blank in the **Included Models** group. Blanks should not be subjected to the random noise because this interferes with the ΔH variation, causing an incorrect distribution.

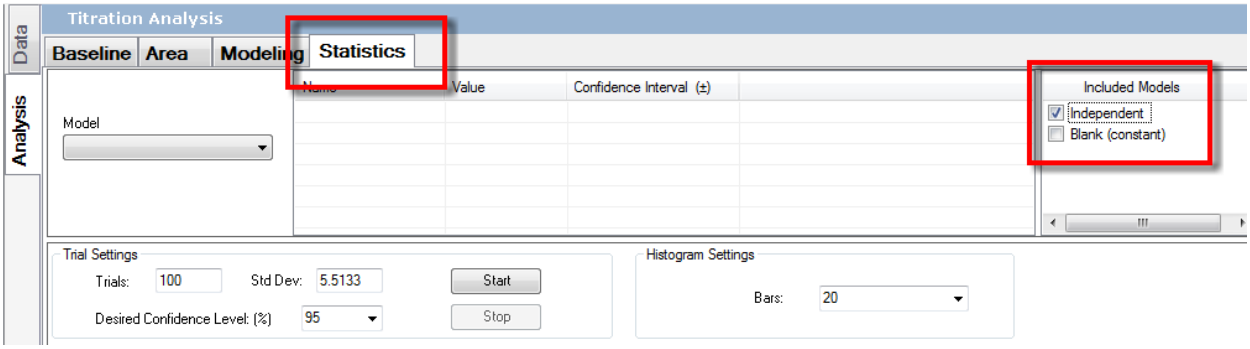


Figure 79 Statistics.

3 Select the model on which the statistical study is to be performed and an appropriate number of trials (typically 100 to 1000) and click **Start**. The figure for **Standard Deviation** was carried over automatically from the **Modeling** page and does not need to be entered manually. The **Desired Confidence Level** and number of bars in the histogram can be adjusted either before or after running the trials. The trials can be halted with the **Stop** button; in this case the histogram and confidence levels will be adjusted according to any trials that have completed.

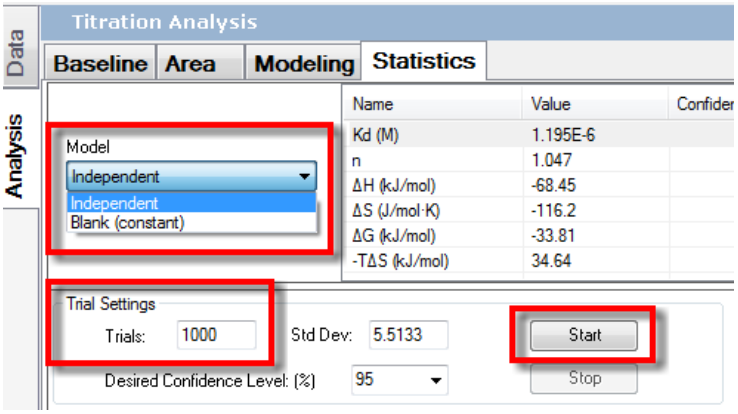


Figure 80 Select number of trials.

4 Select the desired **Confidence Level** (68.27%, 90%, 95%, or 99%), the number of bars (10 to 50), and a parameter from the list in the upper group. The histogram will be displayed in a chart, and the confidence level will be displayed in the graph table and also in the table near the top of the window. The confidence level % and the number of bars can be changed at any time. The greater the number of trials, the closer the distributions of the parameters will be to the expected Gaussian shape.

NOTE: If anything is changed in the **Modeling** tab, the **Statistics** information will no longer be valid and will be cleared.

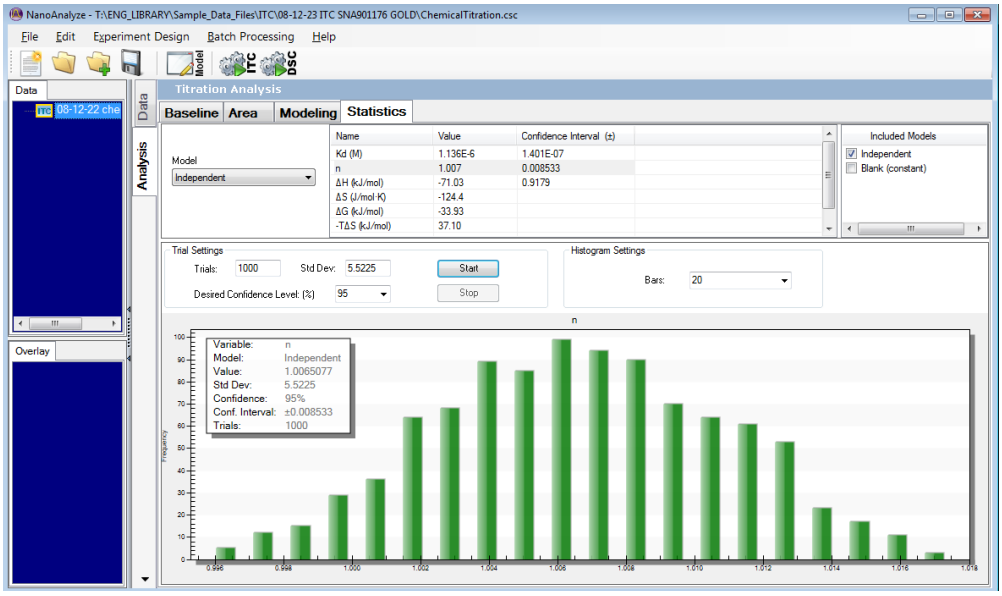


Figure 81 Select the Confidence Level.

5 The **Confidence Intervals** are multiples of the standard deviations of the distributions. An interval of +/- one standard deviation around the mean is assigned a 68.27% probability. Higher levels of confidence are associated with wider confidence intervals.

Number of Standard Deviations	Confidence Interval
1	68.27%
1.645	90%
1.960	95%
2.576	99%

The confidence intervals are based on the assumption that the standard deviations of the parameter distributions have been accurately determined. This is equivalent to an assumption that the observed distribution closely approximates the Gaussian function. However, this will not be a good assumption for small numbers of trials. The following figures illustrate examples that resulted from running 50,000 and 30 trials, respectively. Note that there is a significant bias in the case of the 30-trial example.

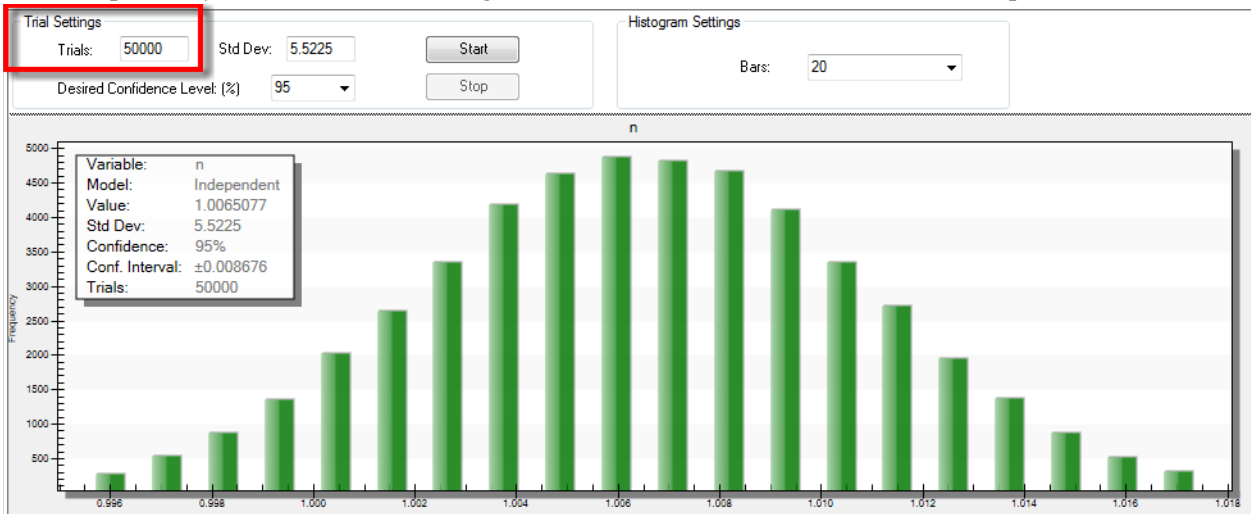


Figure 82 Confidence Intervals.

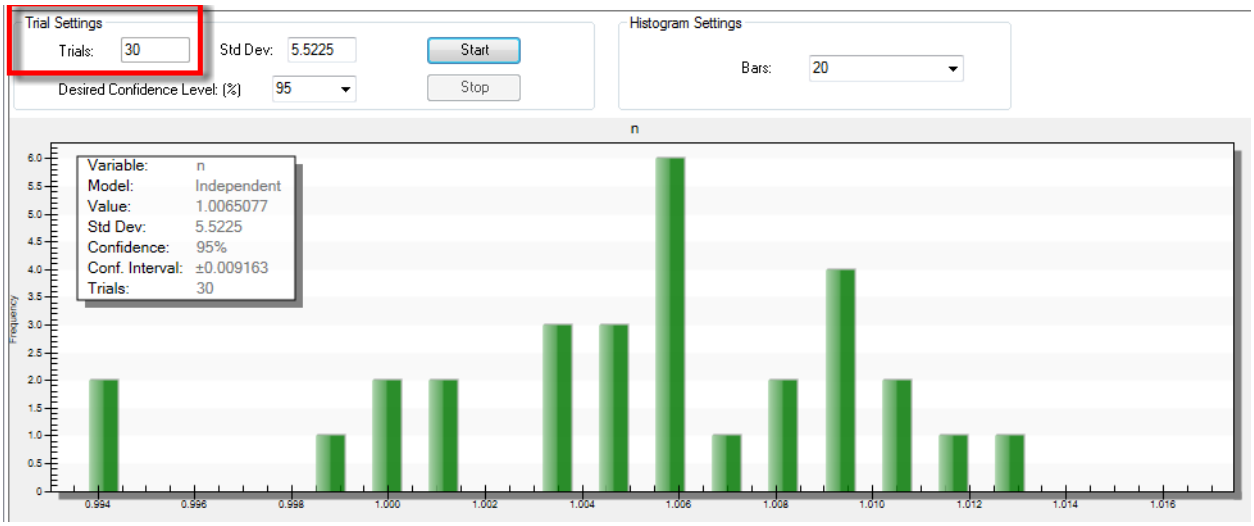


Figure 83 Confidence Intervals.

It is not necessary to produce a perfect-looking distribution. However, the risk of running too few trials is that the calculated confidence intervals might not accurately reflect the true potential for variation. Generally, 100 trials will be sufficient for quick investigations, and 1000 will often be sufficient for more precise work. The figure below illustrates the more consistent results that will be obtained by using a sufficiently high number of trials. Note that with 10 trials the confidence interval results varied by nearly a factor of 6; on the other hand there tends to be little practical benefit to using more than 1000.

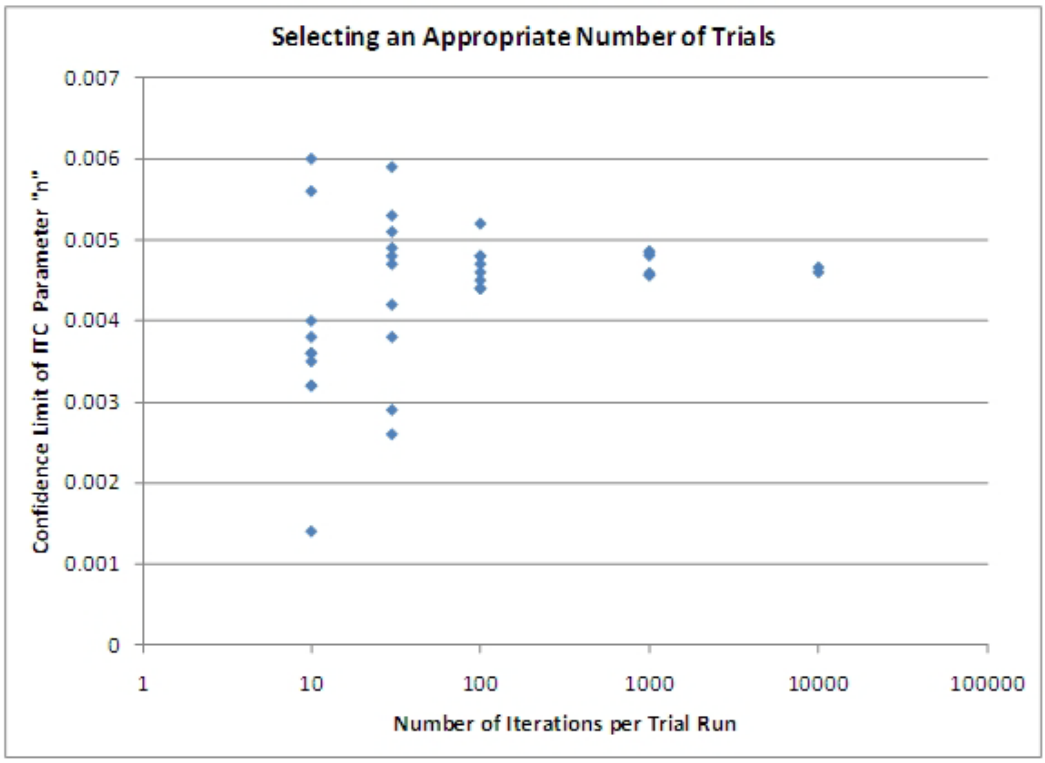


Figure 84 Selecting an appropriate number of trials.

ITC Experiment Design

Introduction

The Experiment Design module is an exploratory tool for optimizing the conditions of ITC experiments in order to maximize the quality of the resulting data. Any ITC model can be incorporated into the simulation and direct controls for all experimental parameters are provided.

An ideal and efficient ITC experiment would generate data from which it will be possible to obtain good values for n, ΔH, and K_a in only one run. This is possible when the typical S-curve of the titration is fully revealed according to these criteria:

- The curve displays a full "S" shape.
- There are multiple injection points populating the low-slope pre-inflection region.
- The inflection point is described well with multiple injections occurring in the central nearly linear section, with a moderate slope.
- There are multiple injections sampling the low-slope post-saturation section of the curve.
- The integrated enthalpy values were large enough to achieve a good signal to noise ratio, which helps insure that the results will repeatable.

The most important decisions to be made before running the titration experiment will be the selections of the sample concentrations. This requires that values are entered for the thermodynamic constants. There may be a need to develop the knowledge of the chemical system in steps. Any *a priori* knowledge of the chemical system can serve as a starting point, or perhaps an initial experiment may have been run but it delivered only partial results. The Experiment Design module makes it possible to refine the next experiment, potentially eliminating multiple trial experiments.

If K_a is high but ΔH is low, there may be mutually exclusive conditions for obtaining good measurements of both parameters within one experiment. A good measurement of K_a may require reducing the sample concentrations, however higher concentrations in a separate experiment may be needed to measure ΔH. Any information learned in either of those experiments can be updated into the model in order to help plan the other.

Using the Experiment Design Feature

To start the ITC Experiment Design module, select **Experiment Design** > **ITC** in the main menu of NanoAnalyze.

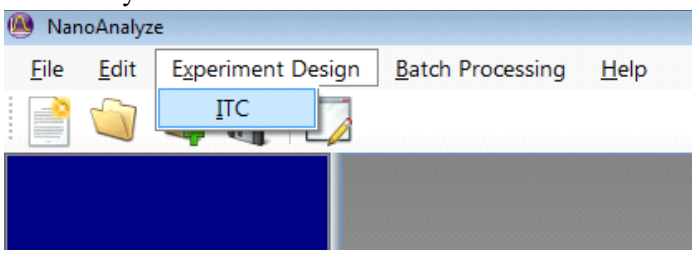


Figure 85 Starting the Experiment Design module.

Click on the indicated control to bring up the model selection dialog box. The Independent binding model will be used for this example. The default settings for the initial default values of the thermodynamic values will be in place, but these can be manually adjusted as necessary.

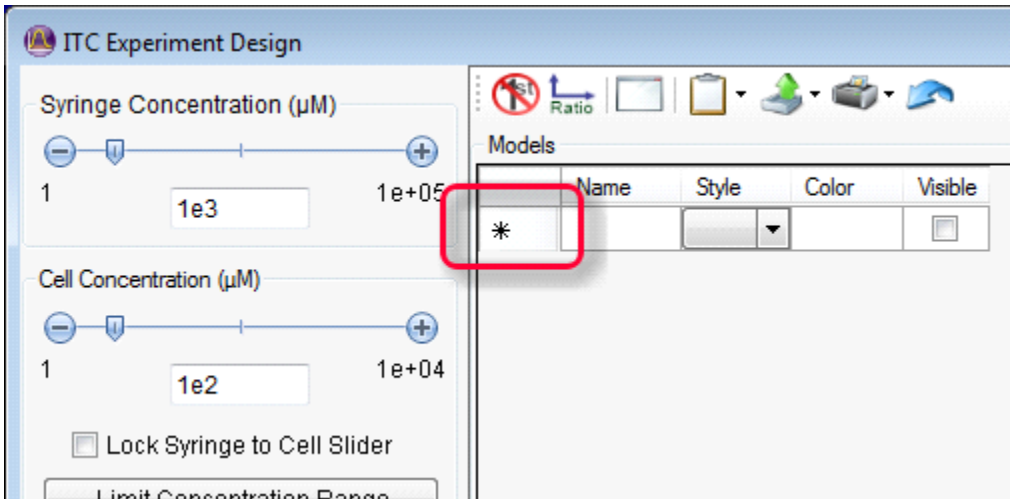


Figure 86 Selecting a Model.

The dialog box is organized into several functional groups as shown in Figure 69, and are described below.

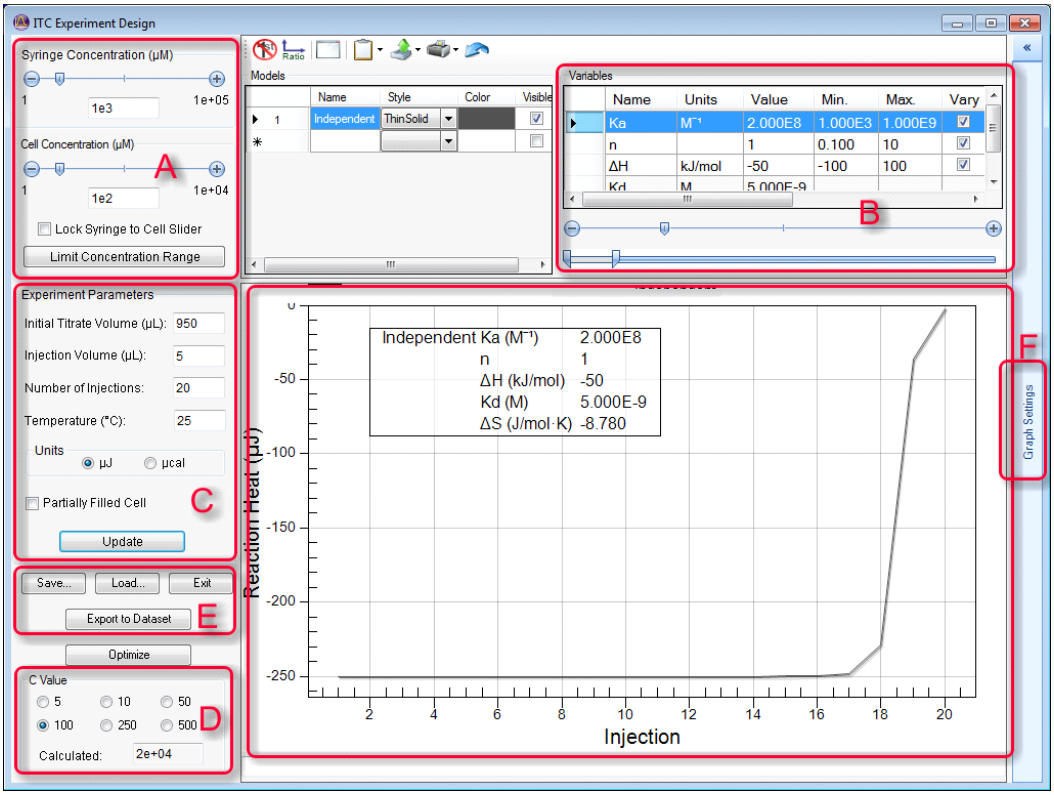


Figure 87 Controls and experiment simulation display.

A Sample concentration adjustment controls.

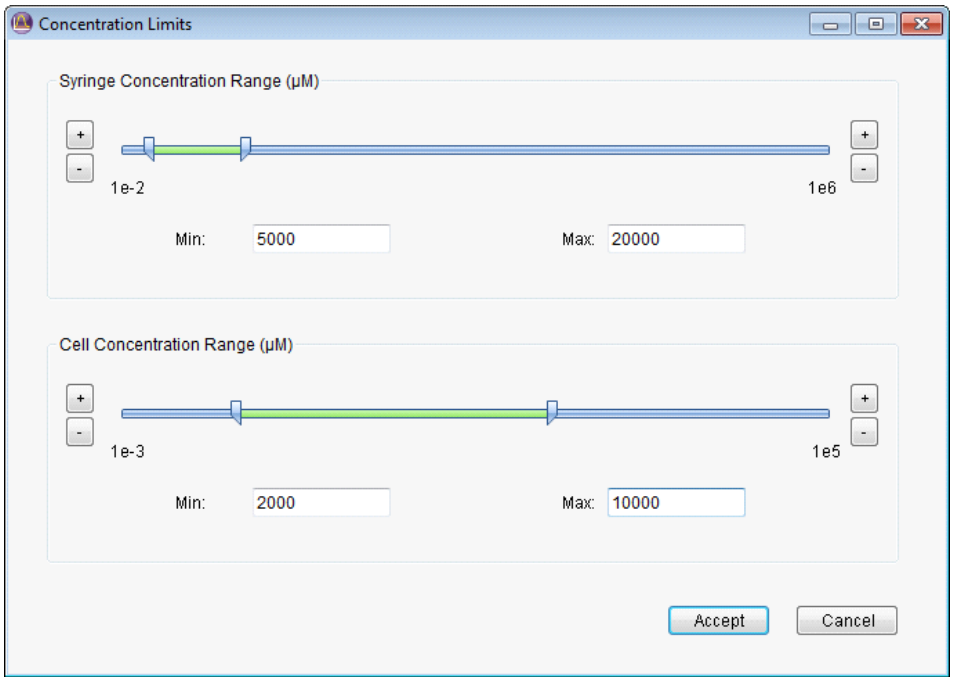


Figure 88 Sample Concentration Limits dialog box.

- Concentrations can be adjusted using the sliders, the "+" or "-" incrementing buttons, or the direct text entry boxes.
- The upper and lower limits of the sliders can be adjusted in the dialog box that is brought up by clicking **Limit Concentration Range**. Use relatively narrow limits in order to obtain fine adjustment increments in a specific region of concentrations.
- When the **Lock Syringe to Cell Slider** box is checked, both concentrations will be simultaneously adjusted (with a constant ratio) when the Cell Concentration slider is moved. The syringe concentration can always be independently adjusted via its slider control. This linked behavior can be very useful in cases of low ΔH and/or high K_a because it clearly demonstrates the trade-off between achieving good enthalpy levels that are well above the instrument noise, vs. achieving several points distributed along a moderate slope in the inflection region. If the slope at the inflection is extreme then K_a would not be accurately determined; only a lower bound would be obtained.
- Changes made to the sample concentrations will be reflected immediately in the graph.

B Thermodynamic variables adjustment controls.

C ITC instrument injection settings group.

- Change the injection settings or the desired enthalpy units in this control group.
- After making adjustments to any of the injection controls, click **Update** to show the current results in the graph.

D Whenever the independent model is loaded there will be selection buttons and indicator reading of the current value of the calculated parameter C, defined as $C = K_a$ x [Mcell] x n. When the value of C is close to 100 the experiment is optimally designed to be able to simultaneously measure n, ΔH and K_a, for the reasons outlined at the beginning of this section.

E Experiment Design File controls. Use these to Save or Load experiment designs.

F The simulated experiment can be copied into the data file tree in the main NanoAnalyze window by using the **Export to Dataset** button.

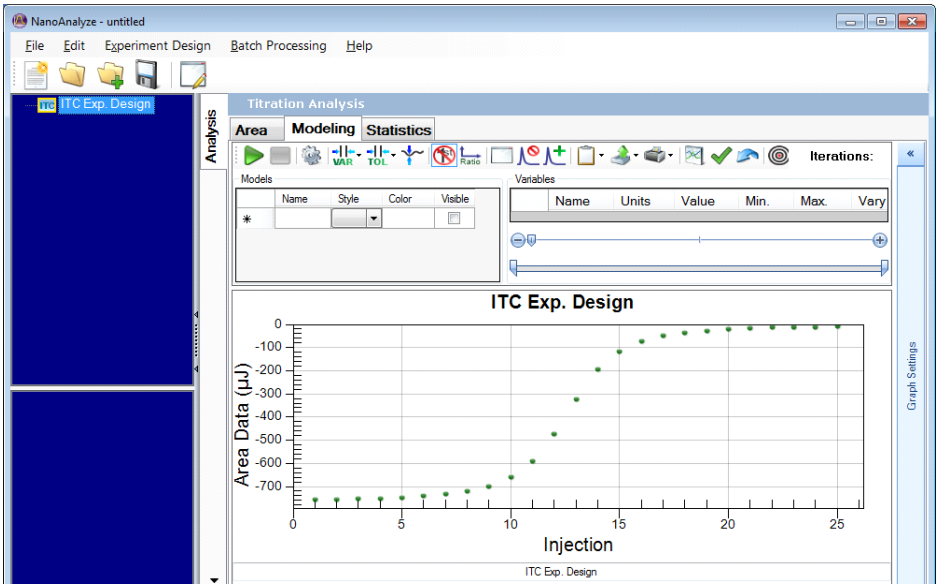


Figure 89 Experiment Design data exported to the main NanoAnalyze window.

G Experiment Simulation graph and the Graph Settings tab.

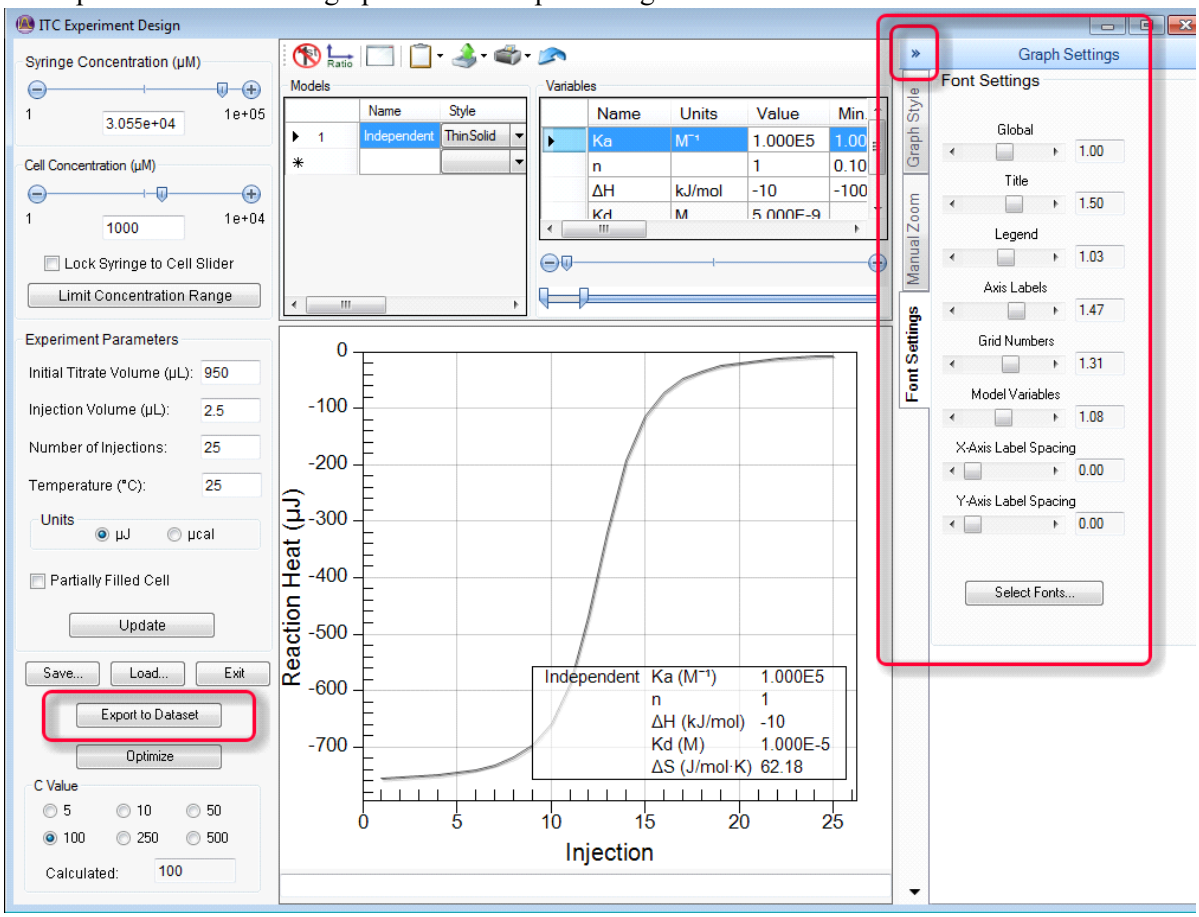


Figure 90 Showing the location of the Export button and the expanded Graph Settings tab.

- The experiment simulation with titration curve and thermodynamic values appears in this section.
- The graph appearance can be adjusted via controls that are hidden by default. They can be accessed by clicking on the **Graph Settings** border. To hide the graph settings, click on the ">>" symbol as highlighted in Figure 72 above.

Independent Model Optimizer

An experiment optimizing feature is available for the Independent binding model. There are many interacting variables which may require several attempts to optimize when using individual manual adjustments. Another alternative is to use the optimizer which makes simultaneous adjustments in order to arrive at a C value of 100. It adjusts the sample concentrations while retaining the current values of the injection settings. If either sample concentration needs to be adjusted outside of the current settings for the slider limits, it will set the ideal concentration and expand the adjustment range as necessary.

There may be external constraints which could make it impractical to use a theoretically ideal optimized design, for example acid-base titrations performed at low concentrations might risk interference from atmospheric carbon dioxide. Other C values can be selected using the option buttons as one way to explore alternative experiment designs.

Pressure Perturbation Experiments

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Introduction

Pressure Perturbation Calorimetry (PPC) is a technique for determination of thermal and volumetric properties of macromolecules. These properties include the partial molar thermal cubic expansion coefficient ($\bar{\alpha}$, Equation 1, [1]) and the relative partial molar volume ($\Delta\bar{V}/\bar{V}$, Equation 2, [2]) of the macromolecule as functions of temperature. These parameters are associated with hydration of the macromolecule [3, 4]. The method consists of measuring the heat effect, ΔQ, from applying a pressure change, Δp, to a solution of the macromolecule. $\bar{\alpha}$ and $\Delta\bar{V}/\bar{V}$ are then calculated by the following equations.

$$\bar{\alpha} = \left(\frac{1}{\Delta V}\right)\left(\frac{\partial V}{\partial T}\right)_p = \frac{\Delta Q}{VT\Delta p} \qquad \text{Equation 1}$$

$$\frac{\Delta\bar{V}}{\bar{V}} = \int_{To}^{Te} \bar{\alpha} dT \qquad \text{Equation 2}$$

The Nano DSC is currently equipped with the necessary components to perform PPC without further modification. PPC is typically performed on a DSC that is capable of increasing and decreasing the pressure on a sample during a DSC temperature scan or during isothermal measurements. During a temperature scan, the pressure change must occur faster than the heating or cooling of the system (3, 4). This note is a short tutorial on the required steps to set-up an experiment on the Nano DSC with DSCRun software and then on analysis of the resulting PPC data with NanoAnalyze software to evaluate $\bar{\alpha}$, which denoted as TEC in the software.

PPC with DSCRun

Turn on the Nano DSC, then open DSCRun and allow the system to stabilize. In the DSCRun software open **set runtime variable** under the **Tools** menu.

On the **Experimental Method** tab, check either scanning or isothermal and enter the temperature limits and scan rate; see Figure 91. If scanning, a slow rate is required because the calculations assume isothermal conditions; see the definition of $\bar{\alpha}$ in Equation 1 (4). If the scan rate is slower than the instrument feedback then it can be safely assumed isothermal during the pressure steps. A rate of 0.1 ºC/min is recommended. This scan rate will typically satisfy the isothermal requirement without scanning so slowly that resolution of the heat rate is unduly sacrificed.

Choose the pressure function to be applied during the experiment. See Figure 91.

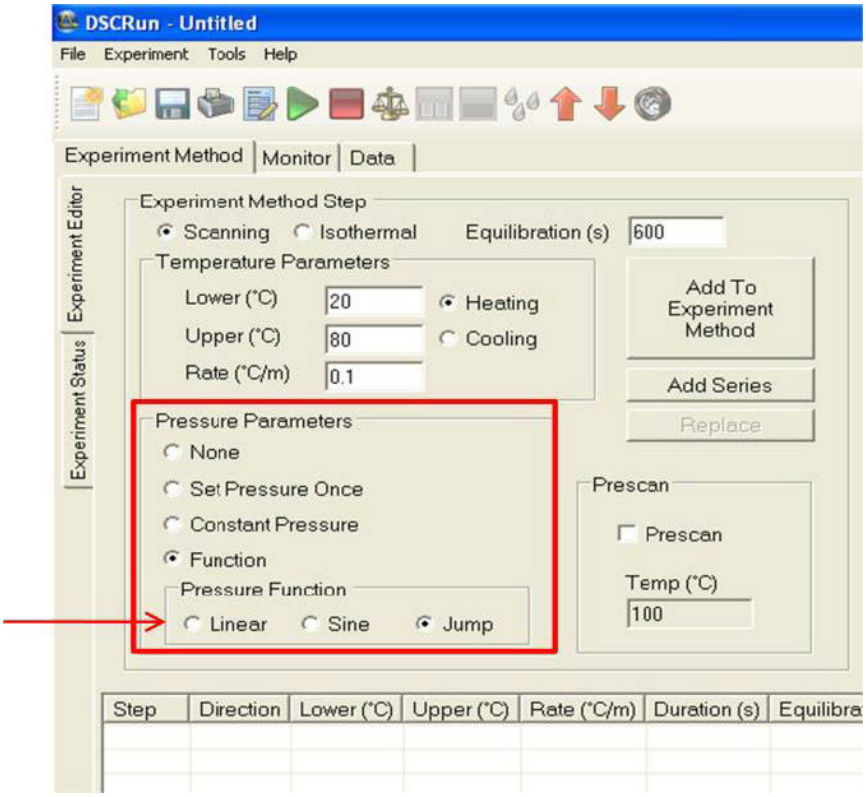


Figure 91 Screen shot of DSCRun highlighting the different pressure parameters available.

Three different pressure control options are available: linear, sine, or ramp (labeled **jump** in the software). The jump pressure function is usually used. Once the desired pressure function is selected and all **Temperature Parameters** are set for the scan, click **Add To Experiment Method**. This opens a dialog box that enables setting the **Jump Pressure Function**.

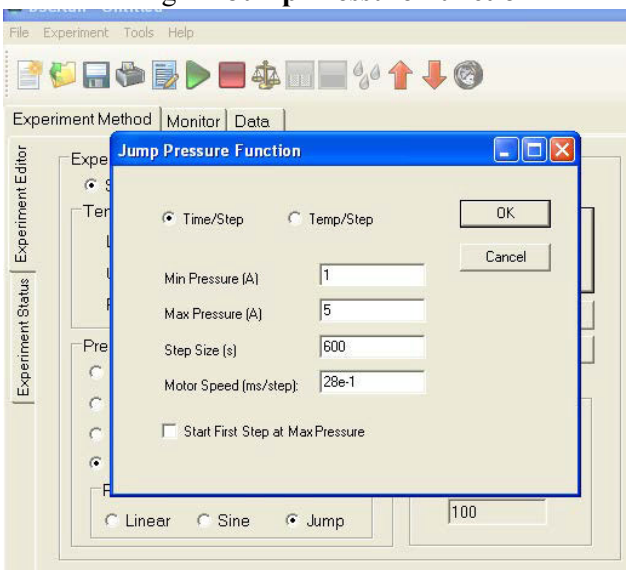


Figure 92 Screen shot of dialog box that opens after a pressure function has been selected. (A indicates atmospheres and s indicates seconds.)

Note that in scanning experiments, pressure can be designated as a function of time or temperature. The term "step" in this box refers to the pressure step. Although the instrument is capable of operating from 0 to 6 atm, relative to ambient pressure, the recommended pressure change is from 1 to 5 atm with a ramp/step time of 600 s (4). The minimum pressure must exceed a pressure that allows boiling at any temperature during the scan. For most experiments, the default motor speed of 28 x 10^{-1} ms/step is sufficiently slow to satisfy the requirement of isobaric conditions in Equation 1.

Experimental Set-up

A water-water scan can serve as a test experiment to assess any asymmetry between the reference and sample cells. For this test, water is loaded into both the reference and sample cell. The pressure is stepped from 1 to 5 atm, starting at low pressure with time steps of 600 s while scanning at 0.1 ºC/min from 20 to 80 ºC. During the experiment pressure is applied equally to both sample and reference cells, so any deviation of ΔQ from zero is solely due to any asymmetry between the cells.

For typical solutions of macromolecules, a concentration of 10 mg/mL should generate good data. Due to the slow scan rate, concentration requirements for PPC experiments are greater than for a normal DSC scan. The PPC experiment consists of two parts, a background run and a sample run. Background data with buffer in both reference and sample cells should be collected under identical PPC conditions as the sample. Sample data must be collected with the same buffer in the sample cell. The basis for this method and the calculations done in NanoAnalyze are as follows (Equations 3-5).

$$\frac{\Delta Q}{\bar{V}T\Delta p} = \alpha_{measured} = \left(\alpha_{sample} - \alpha_{reference}\right) + \alpha_{asymmetry} \qquad \text{Equation 3}$$

$$\alpha_{asymmetry} = \left(\alpha_{buffer\ in\ sample\ cell} - \alpha_{buffer\ in\ reference\ cell}\right) \qquad \text{Equation 4}$$

$$\bar{\alpha}_{macromolecule} = \left(\alpha_{sample} - \alpha_{reference}\right) = \alpha_{measured} - \alpha_{asymmetry} \qquad \text{Equation 5}$$

$\alpha_{measured}$ is calculated from the data with sample solution in the sample cell and buffer in the reference cell and $\alpha_{asymmetry}$ is calculated from the data with buffer in both cells.

PPC Fitting with NanoAnalyze

The data files from the background PPC run and the sample PPC run can be opened in NanoAnlayze without modification. When the raw data files are initially opened, a dialogue box will open with a prompt: **Analyze pressure data for Experiment Step 1?** (Figure 93). In this context, "step" indicates a temperature scan or isothermal run. This request will be made for each temperature scan or isothermal run if the box at the bottom is not checked.

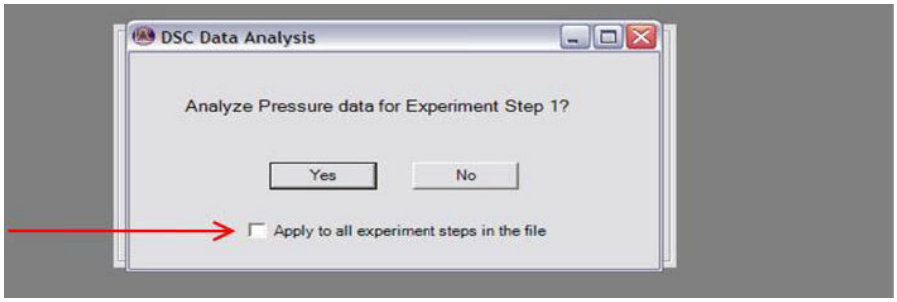


Figure 93 Dialogue box prompt when opening PPC data with NanoAnalyze.

When the files are opened, extra data analysis tabs are automatically made accessible (Figure 94).

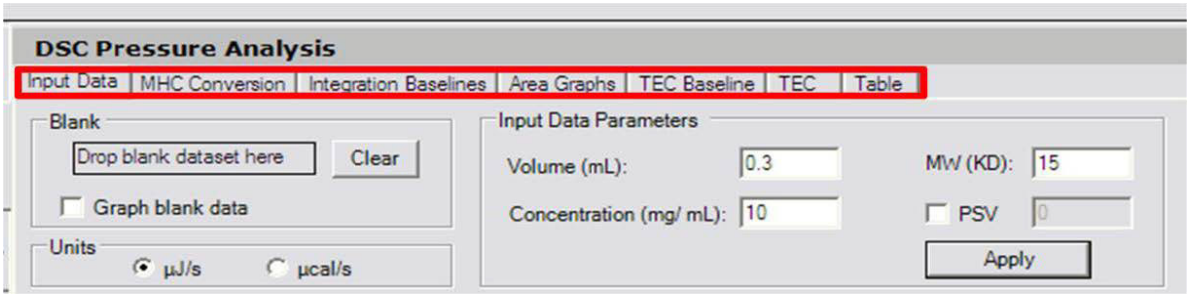


Figure 94 Screen shot highlighting the extra tabs accessible after opening PPC data on NanoAnalyze.

In the **Input Data** tab, several key values must be entered for TEC calculations, i.e., sample cell volume, concentration, and molecular weight in kilodaltons. The data file is loaded with the **Add file** icon. The background data is dragged and dropped into the **drop baseline here** box (Figure 94). MHC (molar heat capacity), the second tab, is not used in PPC calculations. The third tab from the left, **Integration Baseline**, gives the integrated area under each pressure events. The baseline for integration of the heat effects (ΔQ) from the pressure changes can be either a straight-line (default) or a sigmoid (check **Sigmoid Baseline** box) (Figure 95).

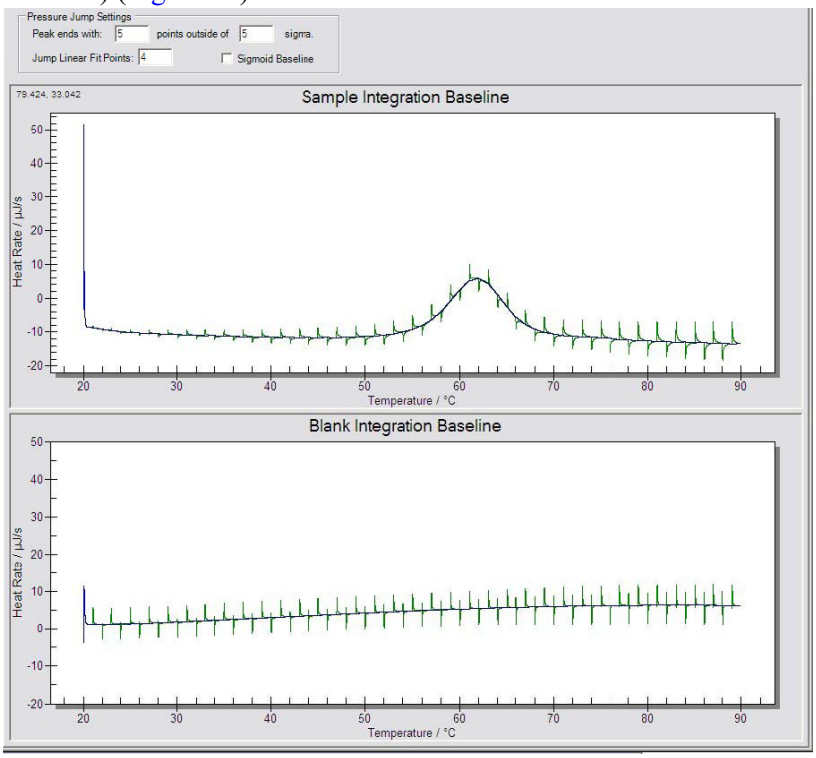


Figure 95 PPC sample and background data in the Integration Baseline window.

In the **Area graphs** tab (Figure 96), background and sample areas are plotted. The baseline area data can be fit to a polynomial, labeled blank fit order in the software (as is the case with TEC Baseline and TEC tabs).

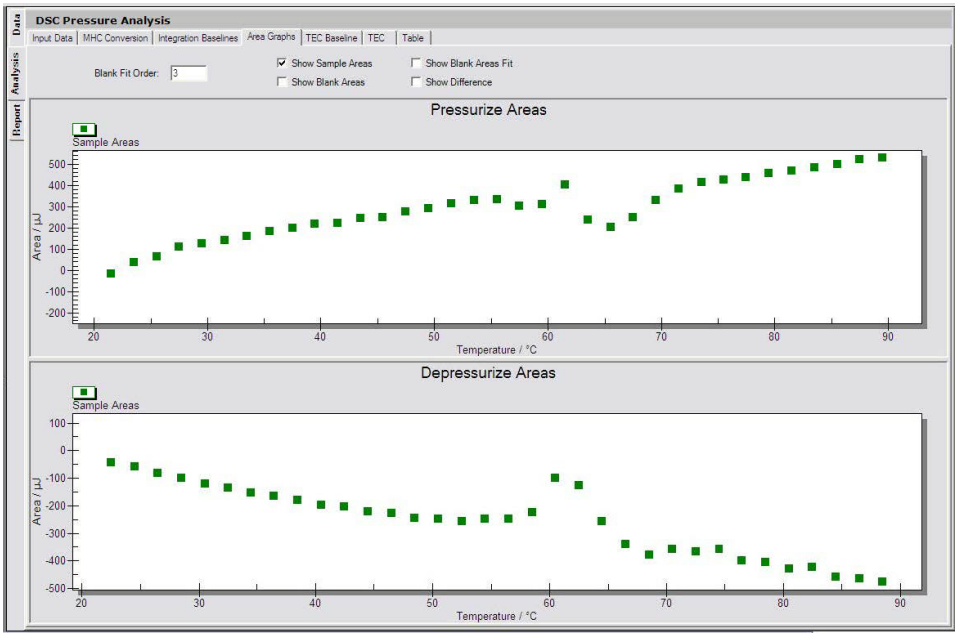


Figure 96 Area graphs window for a sample.

The **TEC Baseline** is used for calculating α for pure liquids, and is not used for calculation of $\bar{\alpha}$ for solutes.

The **TEC** tab plots the thermal expansion coefficients in the top window (Figure 7) and the integrated area in the bottom window. This integrated area is the relative change in volume ($\Delta\bar{V}/\bar{V}$), according to the relationship of Equation 2 (3).

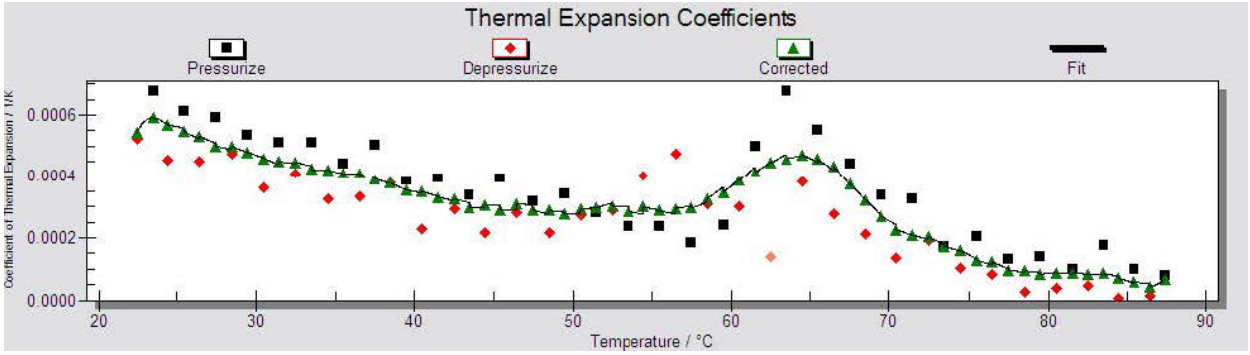


Figure 97 The TEC (α) for a sample.

Numerical values for all of the thermal expansion data are displayed in the **Table** tab and can be copied to a clipboard and imported to another program if further manipulation is desired.

References

1 Randzio, S.L. "Comments on "volumetric studies of aqueous polymer solutions using pressure perturbation calorimetry . . . " [Macromolecules 34 (2001) 4130]" *Thermochimica Acta* 2003 398, 75-80.

2 Mitra, L.; Smolin, N.; Ravindra, R.; Royer, C.; Winter, R. "Pressure perturbation calorimetric studies of the solvation properties and the thermal unfolding of proteins in solution-experiments and theoretical interpretation" *Phys. Chem. Chem. Phys.* 2006 8, 1249-1265.

3 Rosgen, J. "Pressure-Modulated Differential Scanning Calorimetry: Theoretical Background" *Anal. Chem.* 2006 78, 991-996.

4 Dragan, A.I.; Russell, D.J. Privalov, P.L. "DNA Hydration Studied by Pressure Perturbation Scanning Microcalorimetry" *Biopolymers* 2008 91, 95-101.

*Importing Calorimeter Data into NanoAnalyze From *.txt Files*

Calorimeter data in the form of text files can be imported into NanoAnalyze for analysis. The Import function is flexible with regard to character encoding, separator characters, and column layout.

1 In the **File** menu, select **Import Text File**.

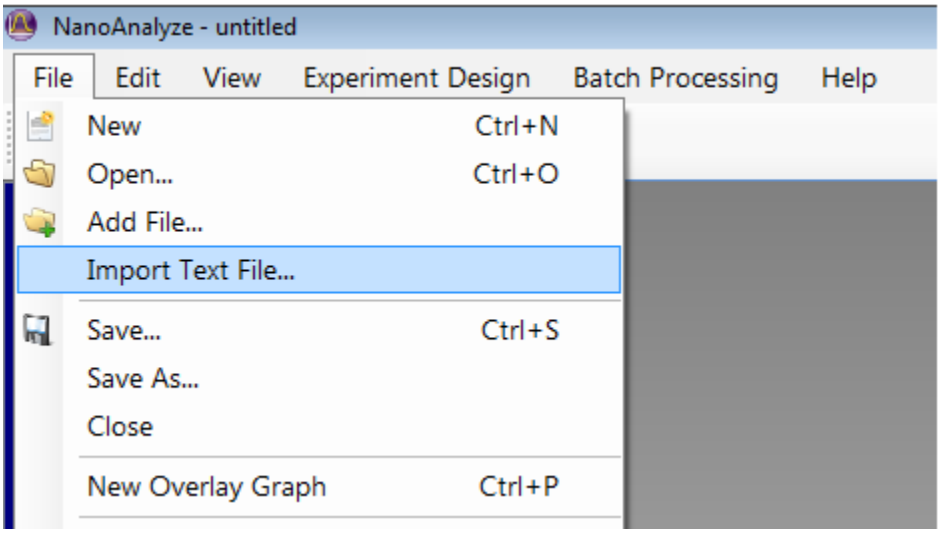
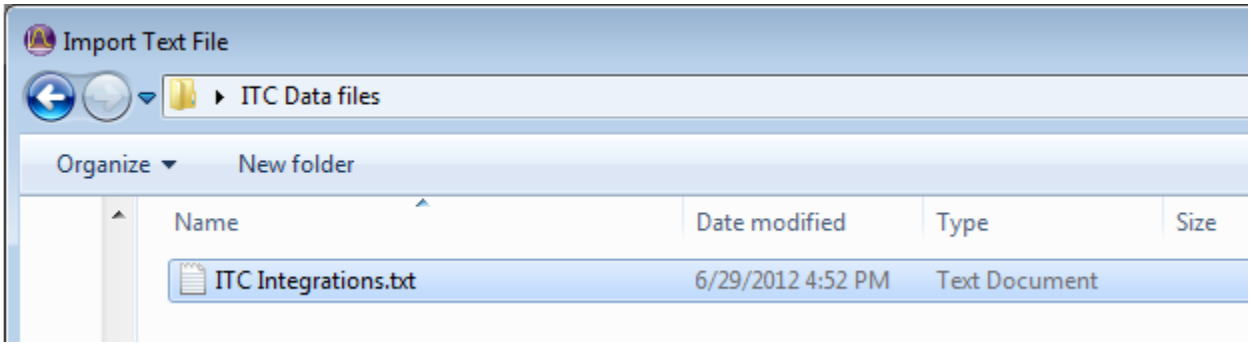


Figure 98 File > Import Test File.

2 Navigate to the folder containing the desired file and select it.



3 Several customizing settings are available to accommodate various options of the format of the text file:

- The **Encoding** selection by default will follow the currently used Regional settings in MS Windows. Other types can be selected if necessary.
- If the text contains more than one column you will need to select the appropriate Delimiter. There is typically a space or other character to separate one data field from the next. Each logical data record such as the data from one point in time, or associated with one integration must appear on its own line. Each line may contain more than one data type, for example a time stamp, a heat rate, an injection quantity, etc.
- The **Analysis Type** must be selected according to the type of experiment.

- Each **Analysis Type** has options for the categories of data that may be present.

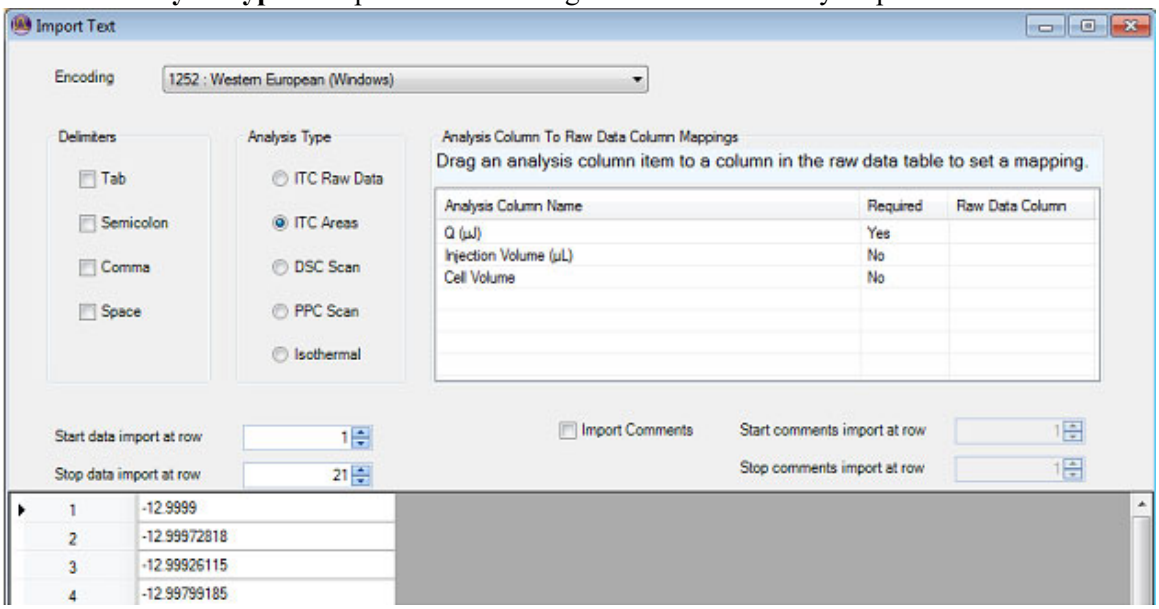


Figure 99 Import text options.

In the following example, integrations from an Isothermal Titration Calorimeter are being imported. In this case there is only one column so the Delimiter is not required. **ITC Areas** was selected. The data to be imported are heat rates, therefore the Analysis Column Name should be selected as **Q (µJ)**. Note that this is a Required field for this data type. If Injection Volume or Cell Volume data are present they may also be imported at this time.

Each column to be imported must have a name assigned. Select the appropriate line from the Analysis Column Name, and drag and drop it onto the column in the data table at the lower left of the dialog box. The text column is highlighted in blue when this has been completed.

If the file contains a header above the data table or any trailing text below the table, adjust the row numbers as appropriate in the fields for **Start data import at row** and **Stop data import at row**. If comments are present in the file they may also be imported; use the start and stop line controls as needed to define what should be imported.

When all data to be imported has been assigned (and if necessary, limited), click **Import**

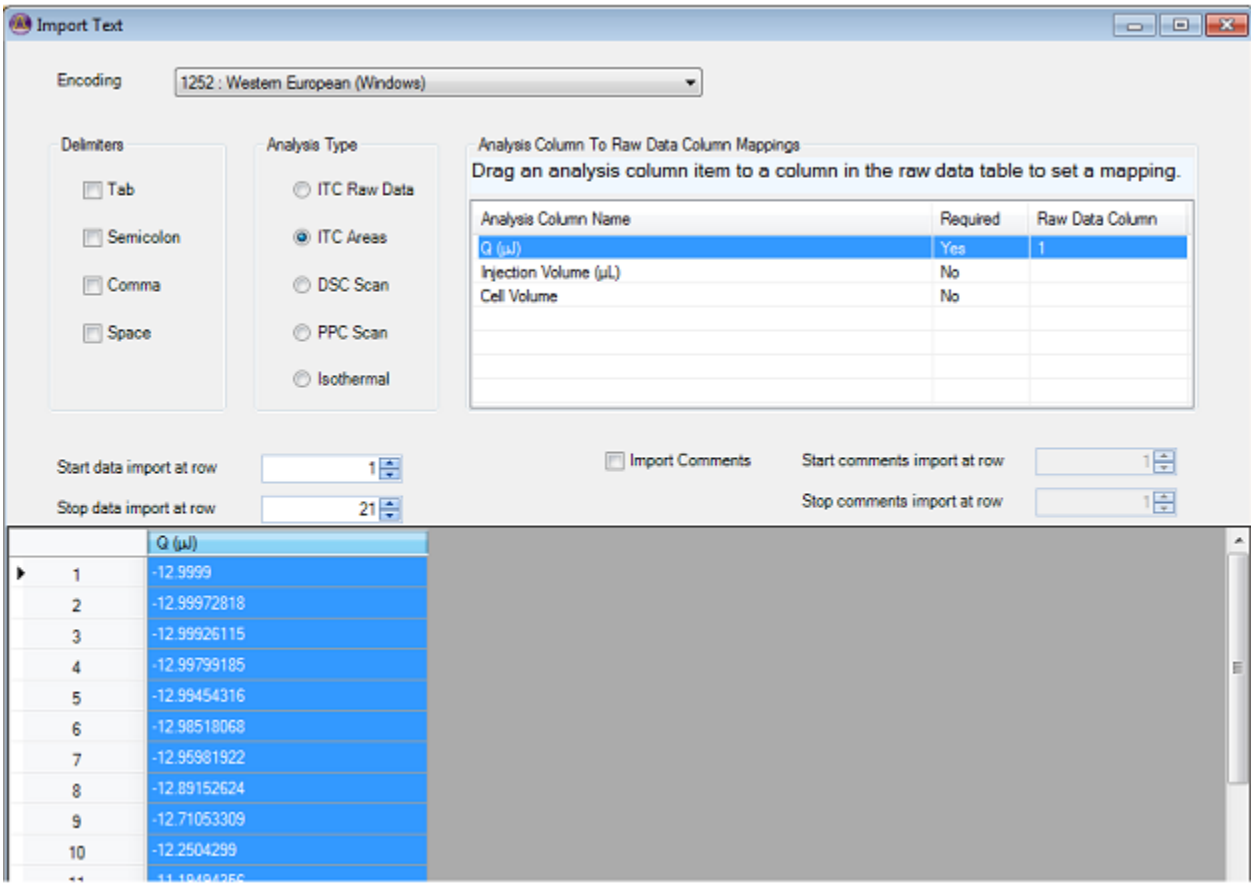


Figure 100

The data will be imported into the appropriate page in NanoAnalyze according to what type of information is represented. Raw data would go into the Data tab. In this case ITC integration areas were imported into the Analysis/Area tab.

Note that some data fields required for Modeling might not be present but they must be filled in before proceeding. Yellow highlights indicated which fields need attention.

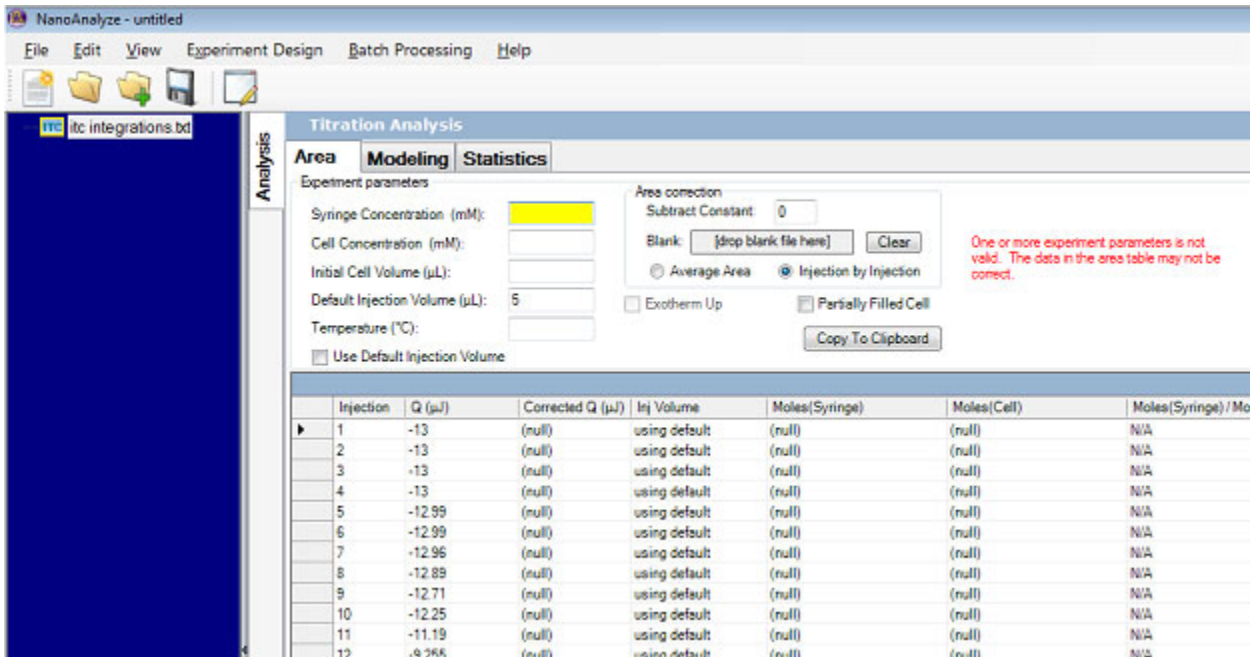


Figure 101 Yellow highlighting indicates fields that need to be filled.

When all required fields have been completed, Modeling may proceed as already described in this Guide.

Overlay Graphs

The Baseline tab also serves as an output graph formatter using the Overlay Graphs feature. Multiple data series graphs can be superimposed if desired, and publication quality charts can be formatted with a wide variety of formatting options.

1 Load a data file and go to the **Analysis** tab (from the left side group) and the **Baseline** tab (from the top group).

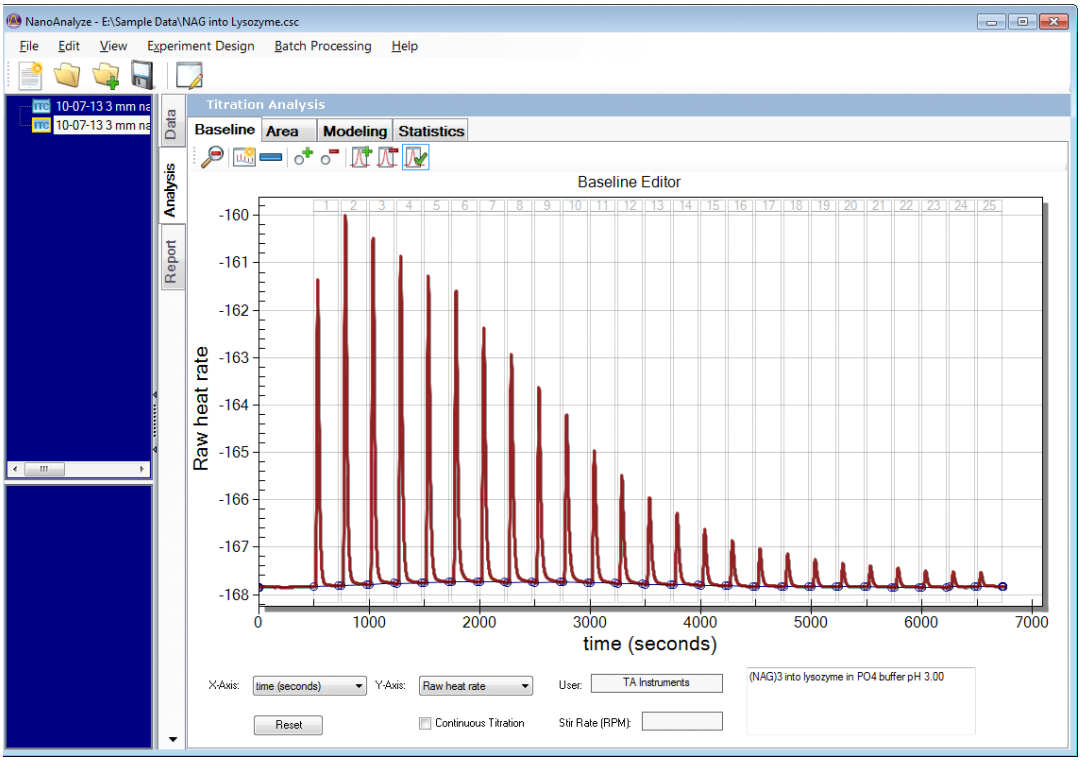


Figure 102 Load a data file.

2 Start an Overlay Graph in one of the following three ways: via **File** > **New Overlay Graph**, **Ctrl**+**P**, or by right-clicking the mouse in the Overlay Graph area at the lower left of the NanoAnalyze window.

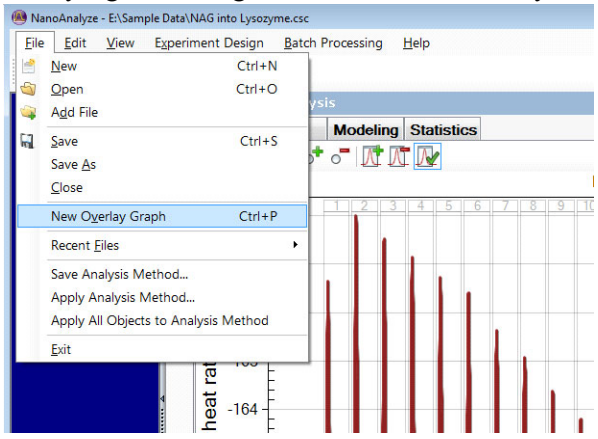


Figure 103 Creating a new Overlay Graph

Creating Publication-Quality Graphs

Drag a data file into the Primary Graph area to the right of the data file tree.

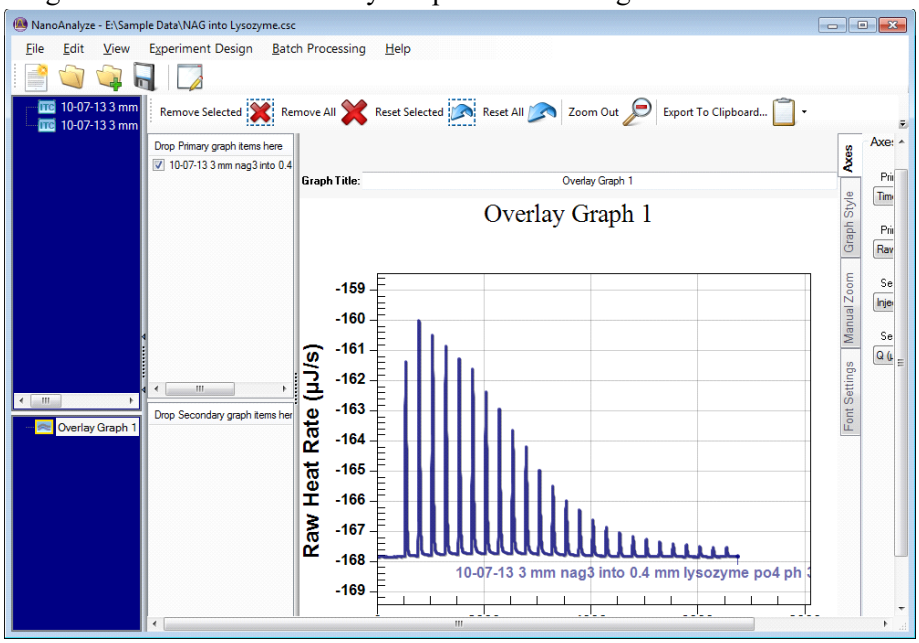


Figure 104 Create the Primary Graph.

A secondary graph can also be created by dragging a data file into the Secondary area. This can be the same data file as the Primary, and can be used to graph a different quantity.

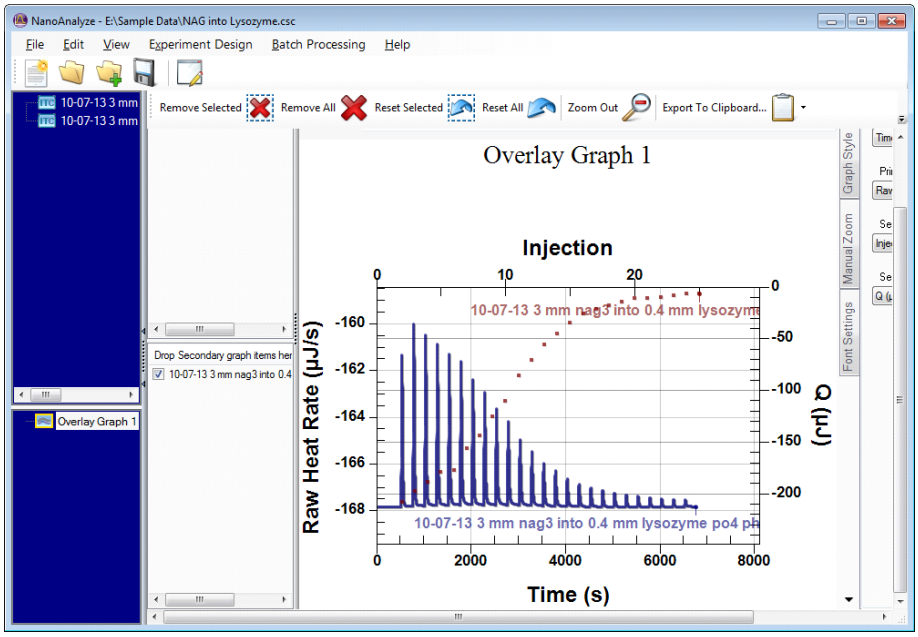


Figure 105 Create a Secondary Graph.

The Secondary graph can be separated into its own chart by using the Split Graph check box in the Graph Style control group, found at the right side of the window.

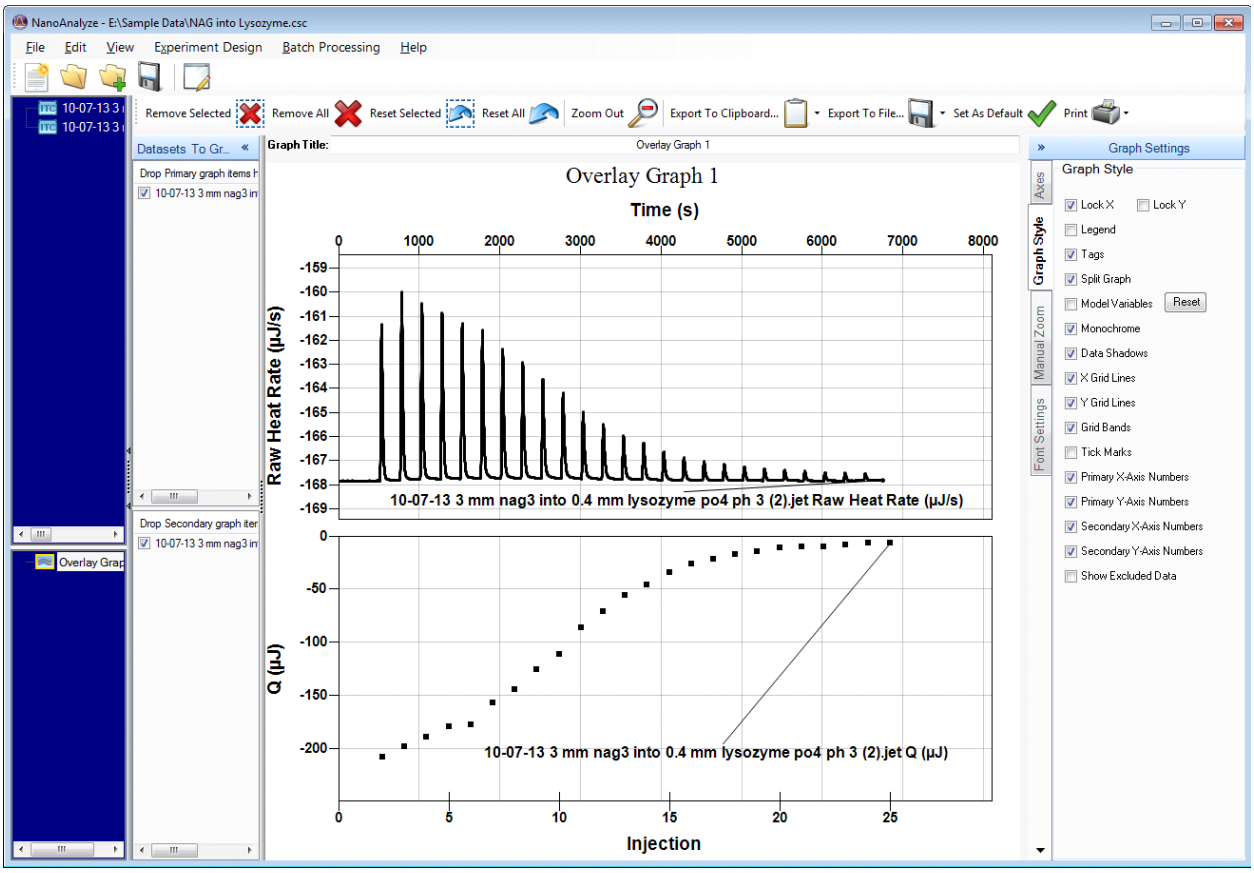


Figure 106 Split Graphs.

The controls for graph formatting are grouped into four tabs at the right side of the window.

The **Axes** tab provides independent controls for each chart, with multiple options in drop-down menus. The **Graph Style** tab contains check boxes to selectively show various chart features. The **Manual Zoom** tab applies the axis limits when the **Manual Zoom** checkbox is enabled. The **Font Settings** tab provides a global font size control along with separate controls for individual text objects.

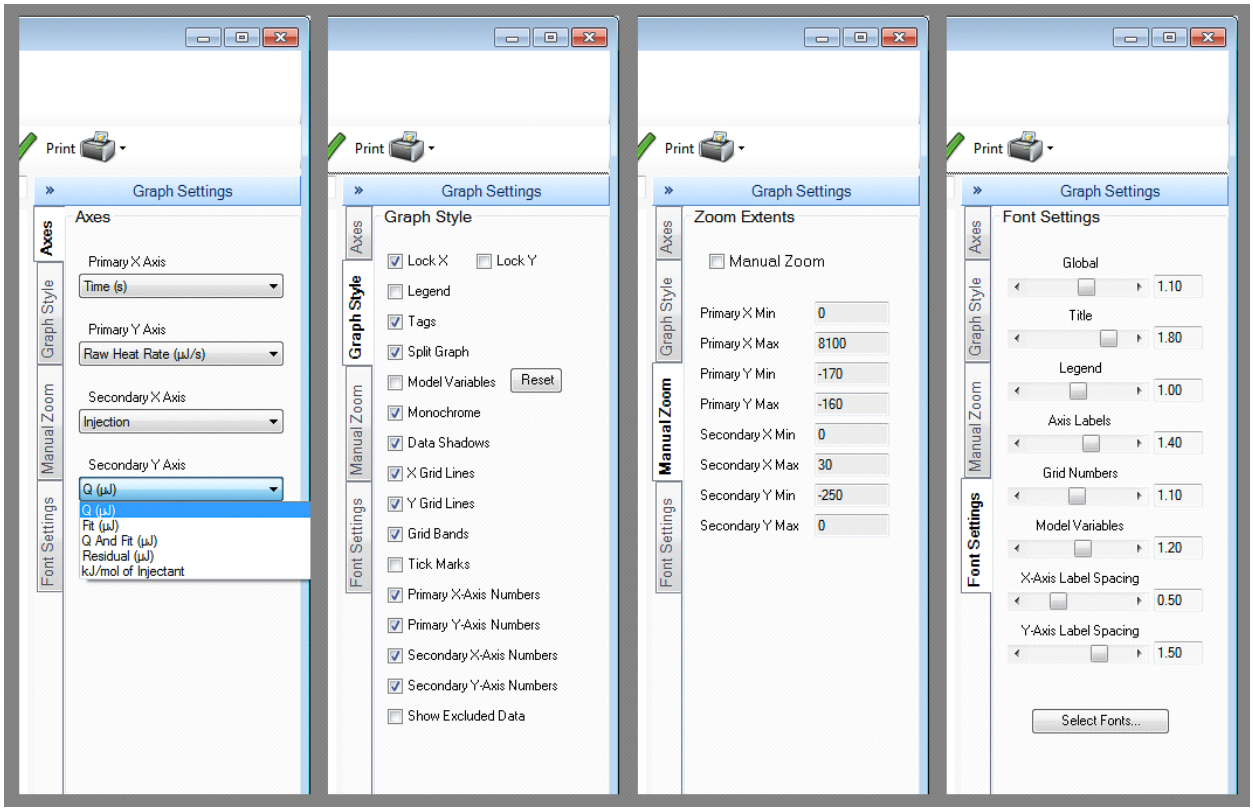


Figure 107 Graph formatting tabs.

Exporting Publication-Quality Graphs

The tool bar at the top of NanoAnalyze contains controls to allow exporting graphs in a variety of formats to either the file system or into the Windows clipboard for pasting into other applications. The export format options include the two scalable image formats EMF and WMF. There are also fixed-resolution raster output formats BMP, JPG, and PNG. Generally the highest quality images are available in the EMF and WMF formats.

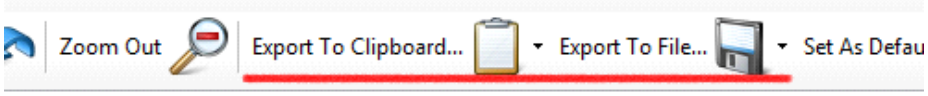


Figure 108 Export Controls.

1 Click on either **Export to Clipboard…** or **Export to File…** and select the desired image format. If you choose to create a file you will be asked to enter the file name and select a folder location.

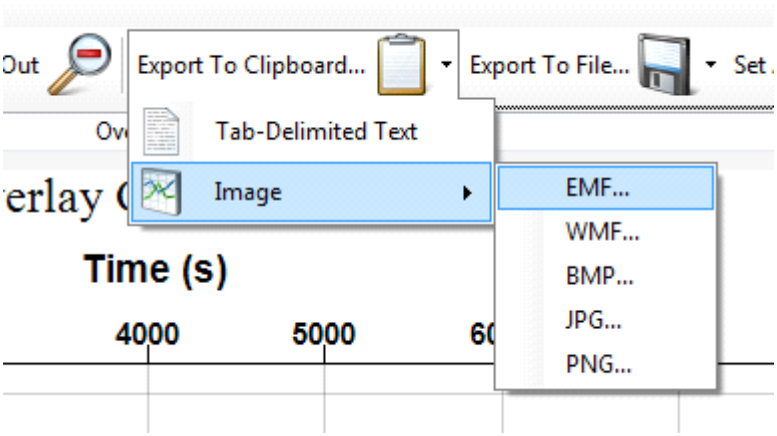


Figure 109 Selecting image export format.

2 A dialog box displays a representation of the graph. The aspect ratio, or ratio of length to height of the image, can be selected. Click **Export** to complete the graph export.

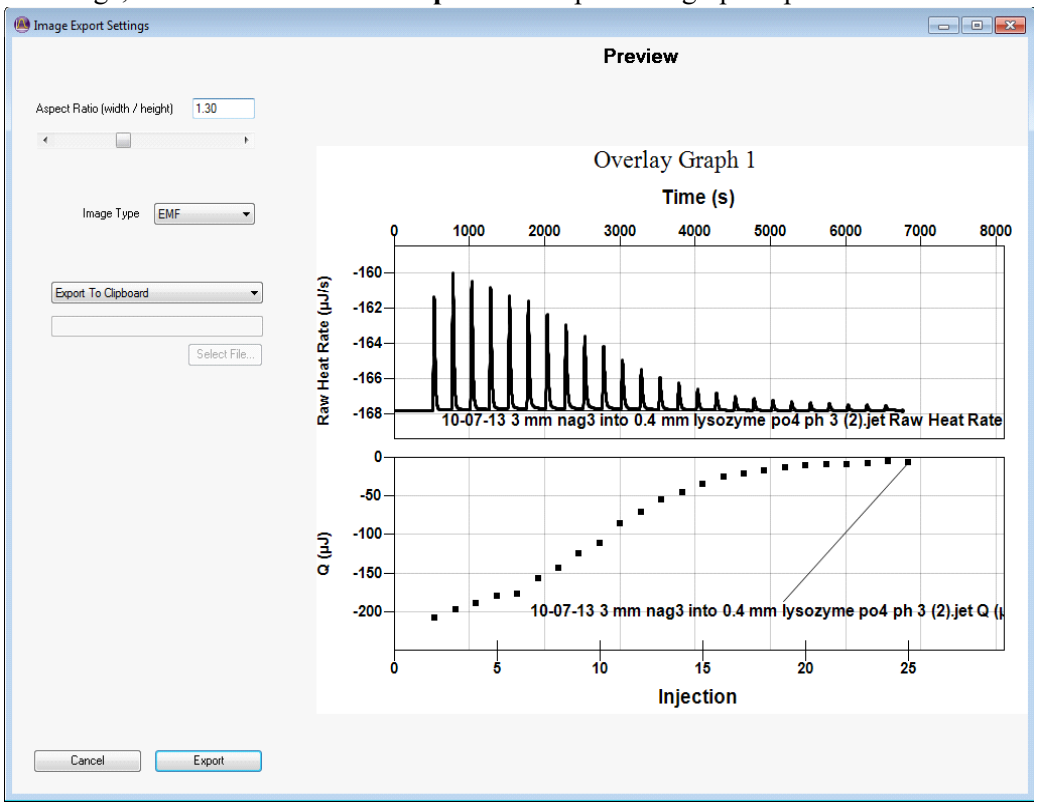


Figure 110 Image Export Settings.

Creating Graphs with Multiple Data Files

Load multiple files that you wish to compare into the file tree, and create a new Overlay Graph. Populate the graph by dragging files from the file tree over into the Primary graph data area.

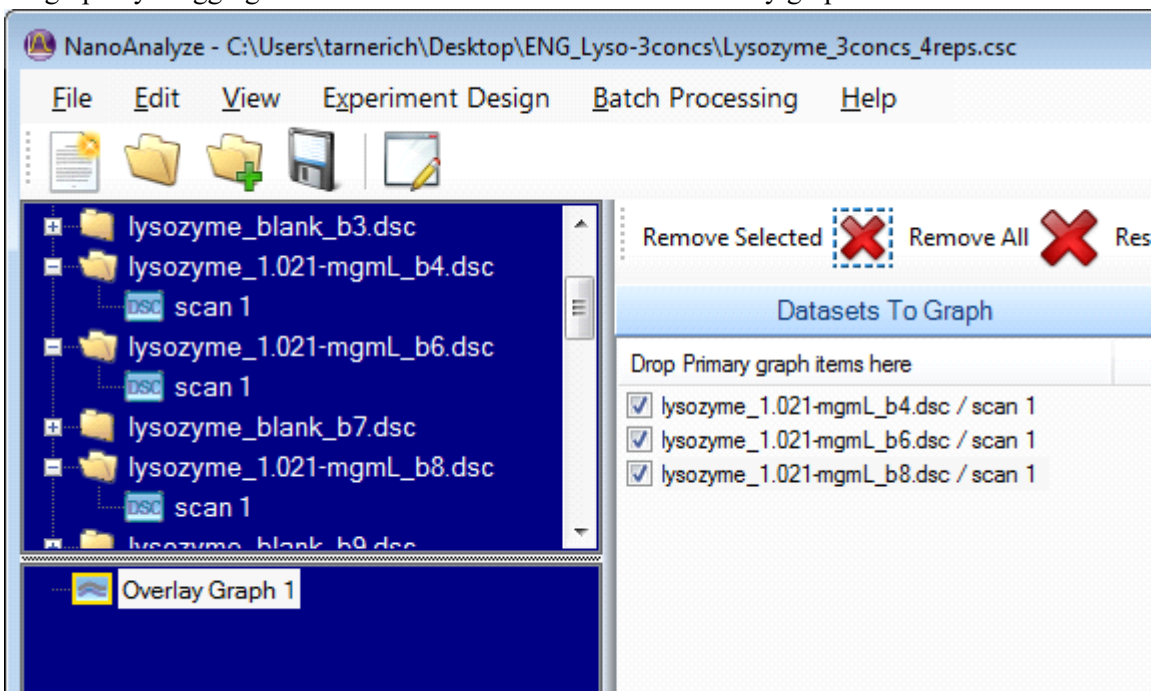


Figure 111 Populating multiple files into an Overlay Graph.

The data traces from each experiment will be graphed together. The files may be manually moved within the graph window to create a separation for visual emphasis. Note the DSC scans cannot be moved horizontally along a Temperature axis. In the Graph Style tab, select or deselect Lock X-Axis or Y-Axis as appropriate. Click on a data trace and drag to the desired position. Handles will appear on the selected data trace.

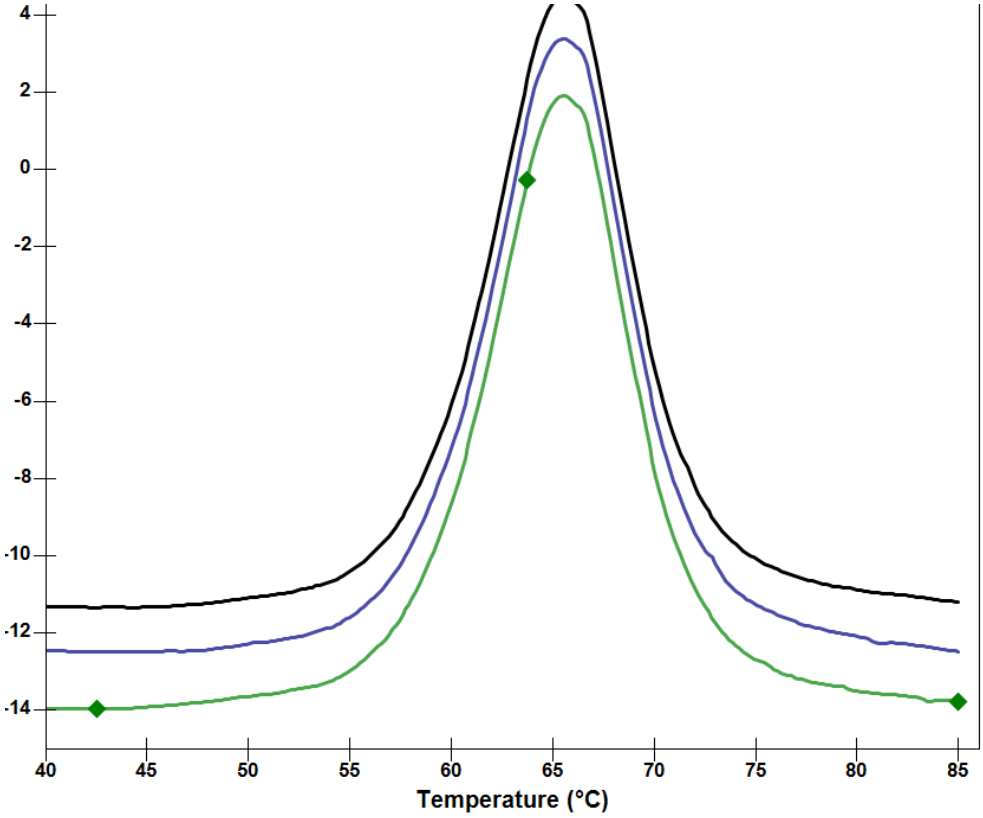


Figure 112 Selected data trace.

Exporting Data

It is frequently necessary to export instrument raw or processed data to external software applications such as word processors or spreadsheets. Data export functions have been provided in most locations within TA Instruments' software applications.

Data Export from a Table

Where data appears in a table (for example, in the NanoAnalyze **Data** viewing tab for a Nano ITC experiment or a Nano DSC scan), place the mouse cursor over the data table and right-click the mouse. A small pop-up menu appears.

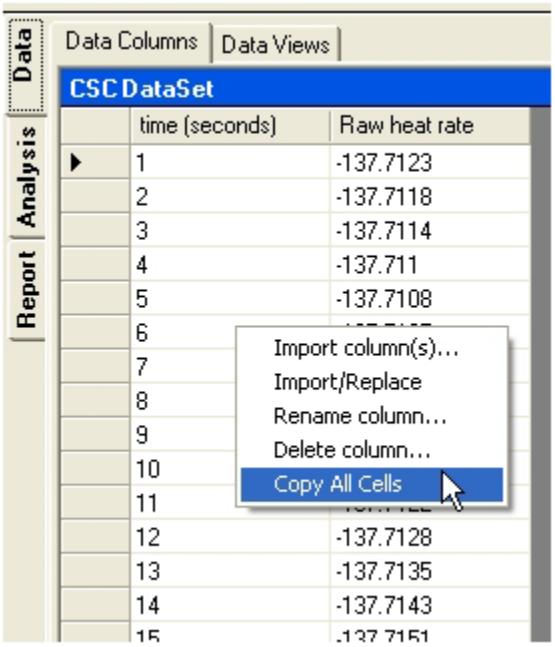


Figure 113 Copy All Cells.

Move the cursor onto and then release the button over the item labeled **Copy All Cells**. An hourglass icon may appear briefly while the data table is copied to the Windows clipboard. When the cursor returns to the normal arrow, the clipboard will contain a copy of the complete table for the entire experiment or scan. In the receiving application, use the mouse to select the desired location to paste the data. The application's **Paste** or **Paste Special** function (or the **Control-V** keyboard combination) will then create a copy of the data in the receiving application.

Copy to Clipboard Function

Some pages support data export via a dedicated control such as the **Copy to Clipboard** button seen in the **Area** tab of NanoAnalyze. The data is copied into the Windows clipboard when this button is clicked, and is ready to paste into a receiving application.

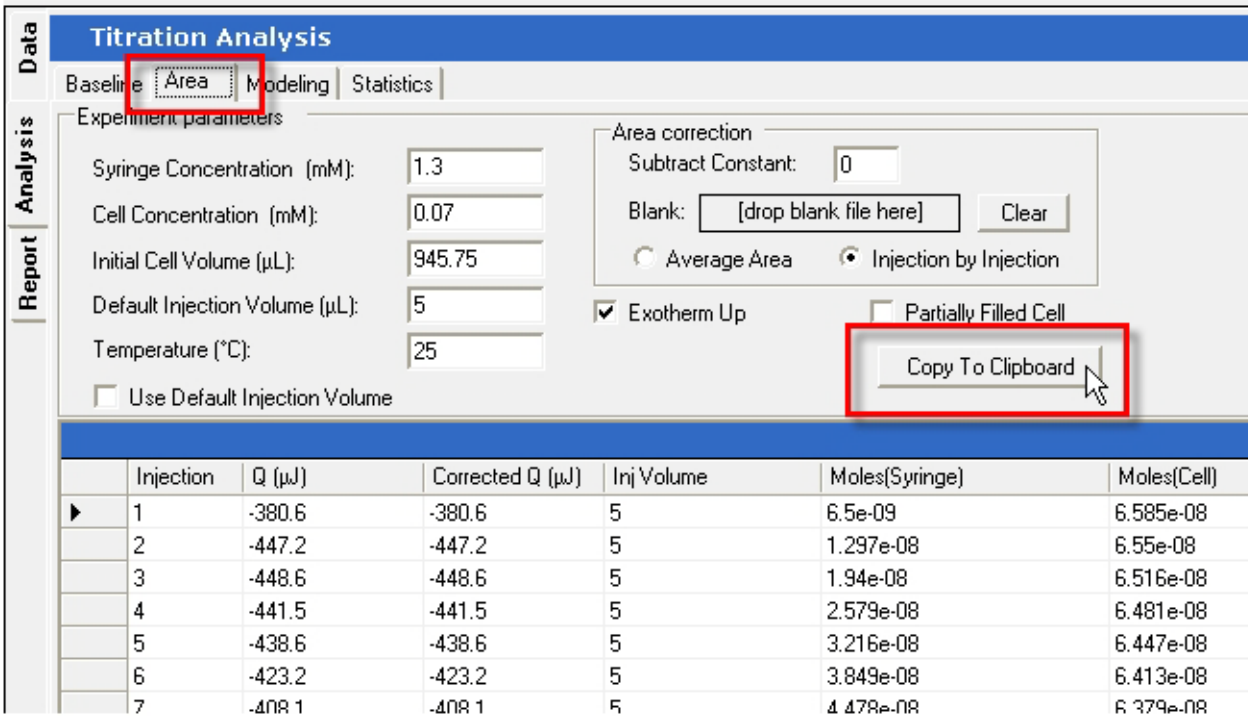


Figure 114 Area tab > Copy To Clipboard.

Export Dialog Function

Pages that display graphs have an export function that is accessed via a pop-up menu within the chart area.

1 Right-click the mouse inside the chart and select the **Export Dialog** item on the menu:

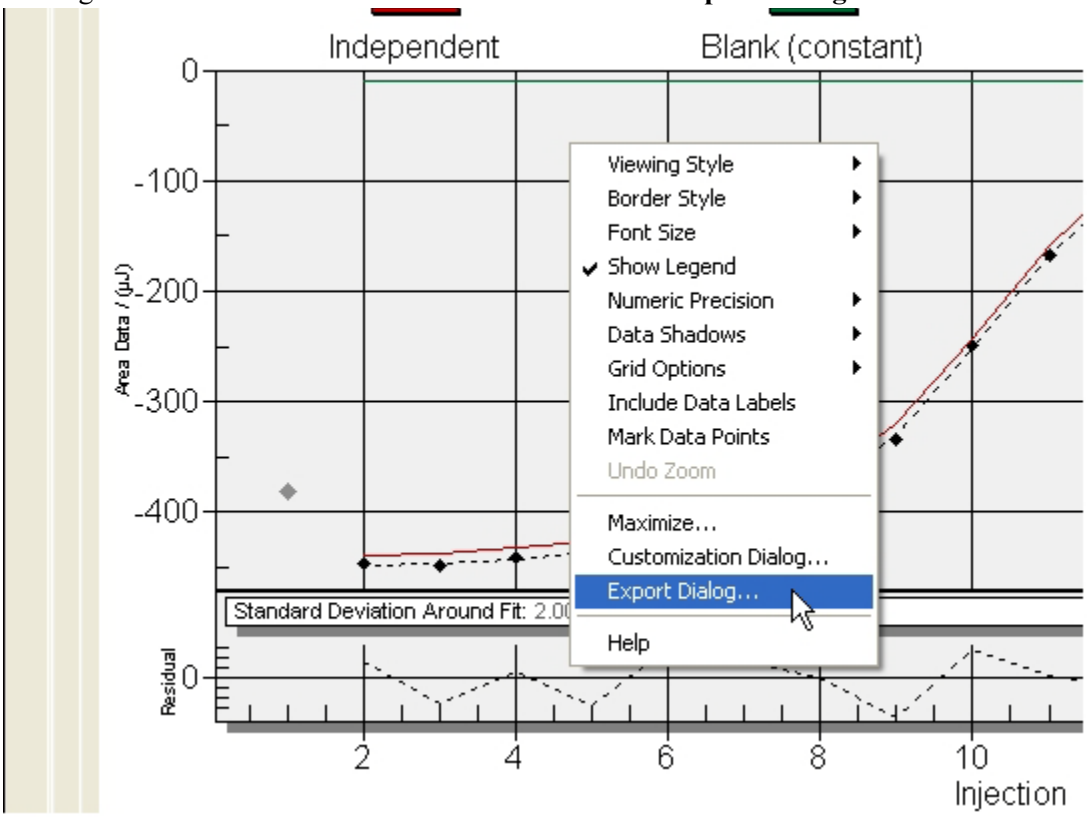


Figure 115 Export Dialog...

2 Select the **Text > Data Only** option and click the **Export** button. (Another available feature is to export an image of the graph using the Metafile, BMP, JPG, or PNG formats.)

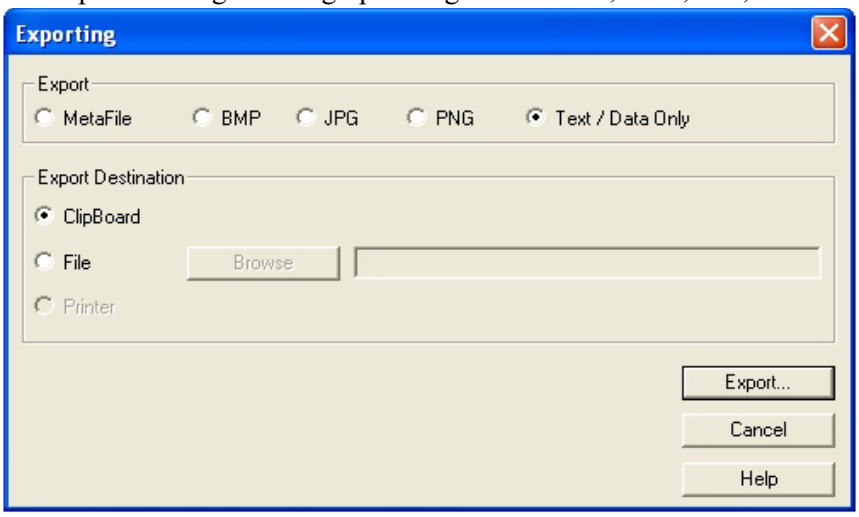


Figure 116 Select Text/Data Only.

3 For a more compact table of numeric results, select **Data** in the **Export What** group. The **List** option in the **Export Style** group creates a vertical array of data (narrow and long), while the **Table** option creates a horizontal array (wide and short). In general it is best to use the **Maximum Precision** option.

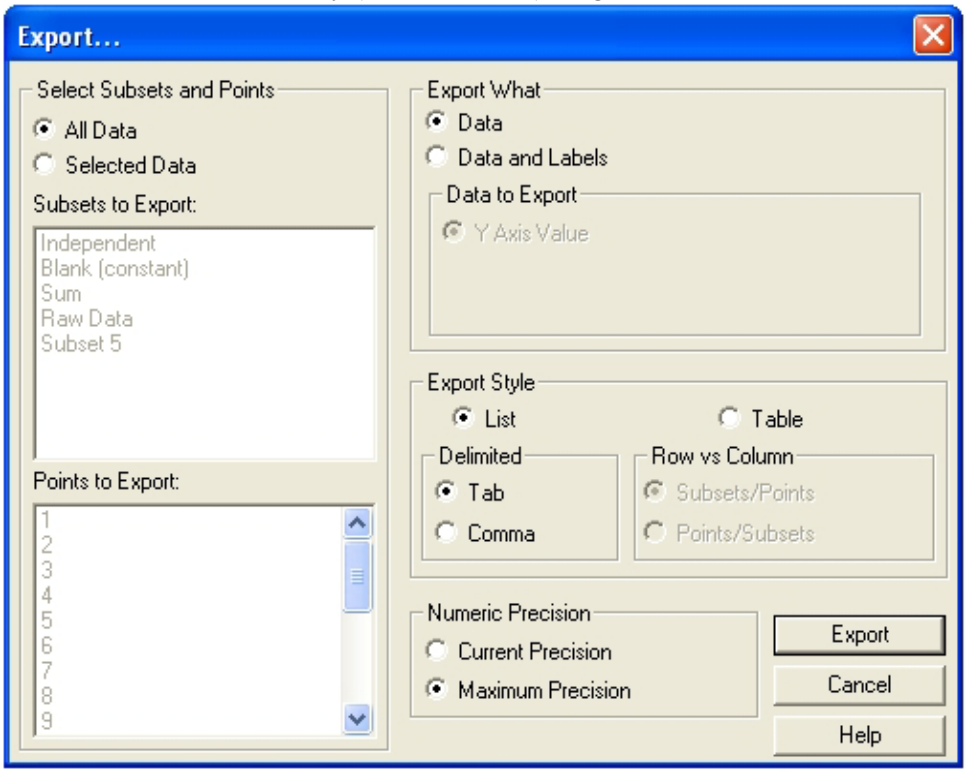


Figure 117 Export... dialog box.

When the data is copied to the clipboard, the dialog box closes.

Display Format Options

The visual appearance of TA Instruments software applications can be altered via functions available in a pop-up menu. Right-click the mouse while inside any window or tab to bring up the menu. There are appearance options for the screen area, plotting style, font, and number formats. Many of these options can be directly accessed from this menu.

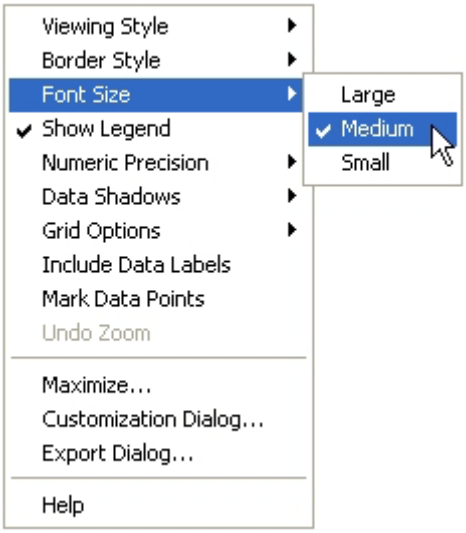


Figure 118 Formatting appearance.

A more complete set of display options can be accessed through the **Customization Dialog** menu item. Right-click on the data graph to bring up the menu:

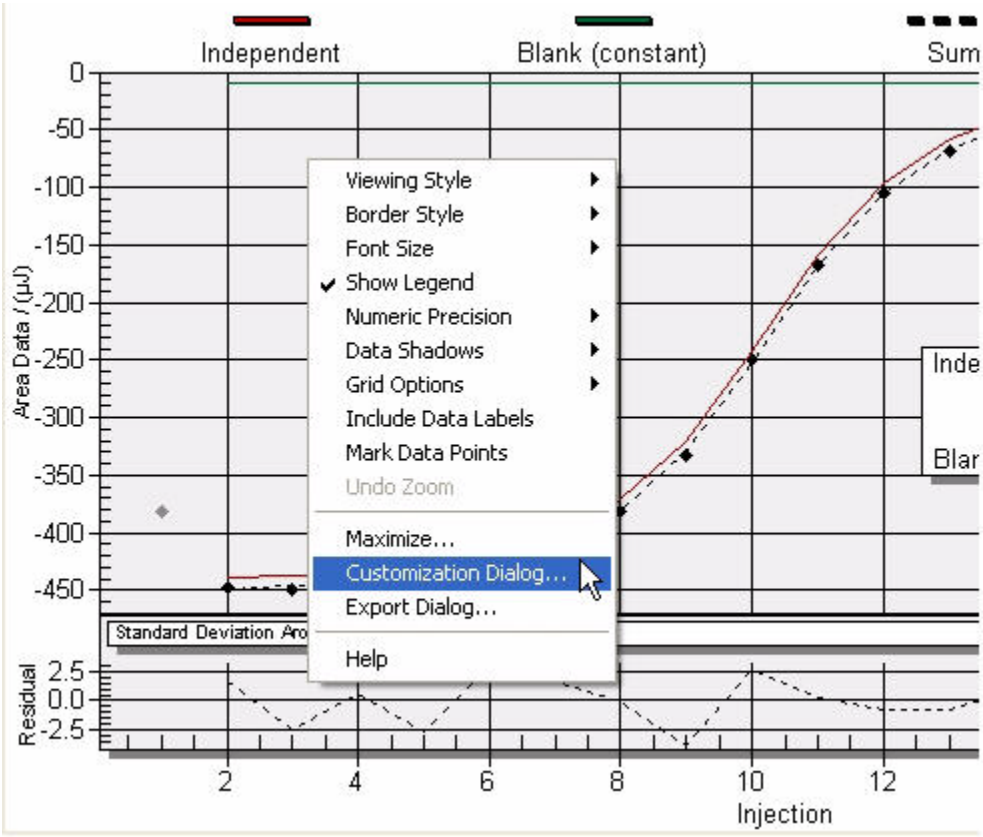


Figure 119 Select Customization Dialog...

The display controls have been grouped into tabs for easy access.

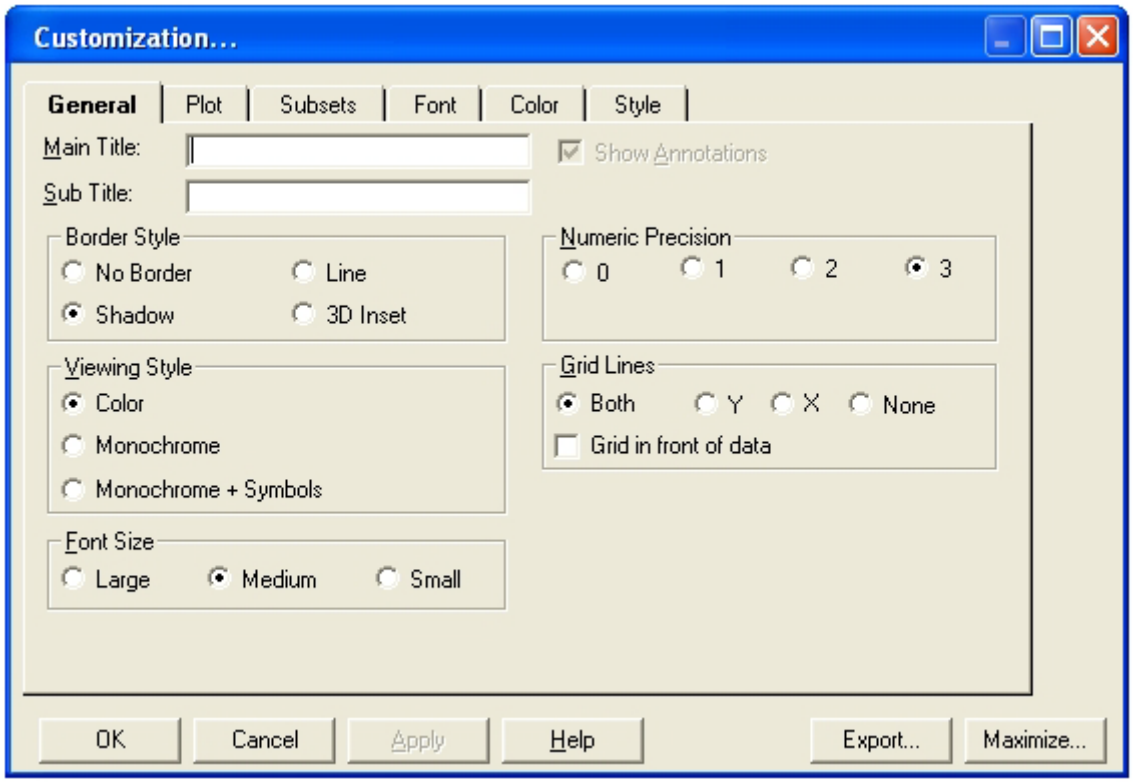


Figure 120 Customization... dialog box.

Appendix: A

NanoAnalyze Reports

Creating the Report

After a data file has been analyzed a customized report can be created.

1 Select the **Reports** tab at the top of the software window.

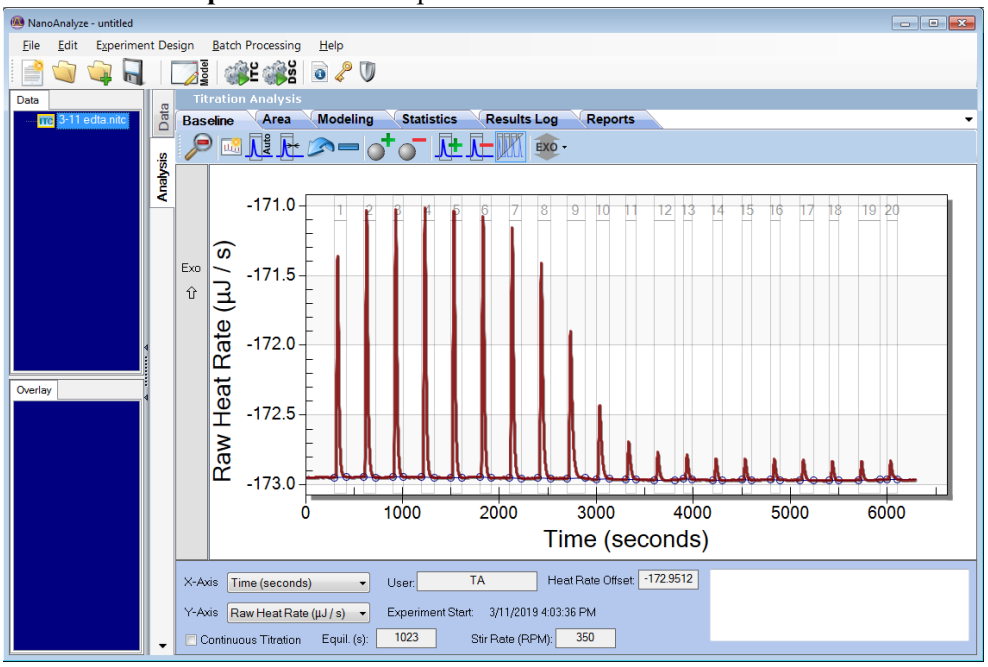


Figure 121

2 Select to **Create Report (Default Template)**, **Create Blank Report,** or **Create Report Using Template File.** Click **Ok**

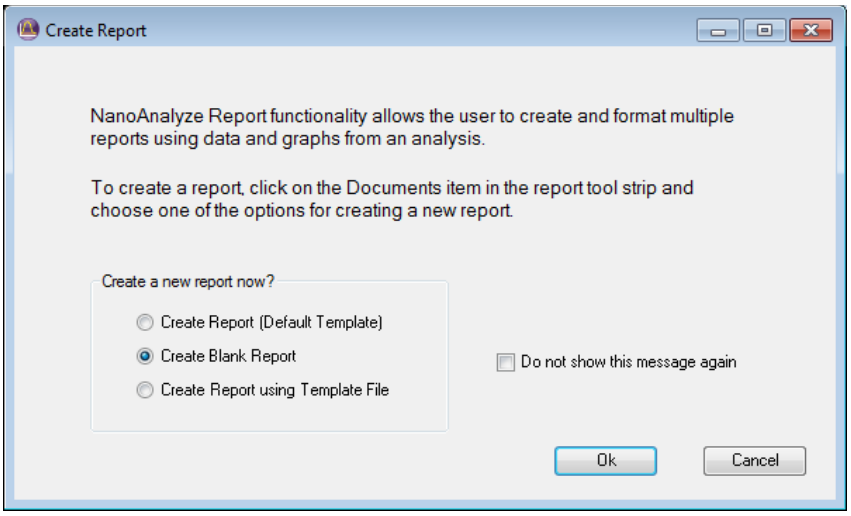


Figure 122

The report file opens based on the selection above. In this example, and empty Reports page displays.

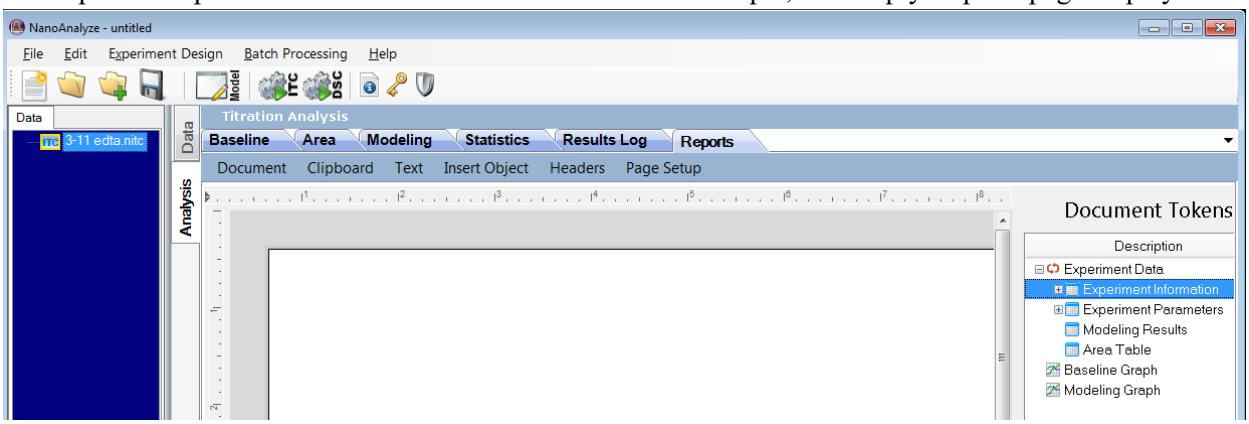


Figure 123

Report Tab

The following functions are available in the Report tab:

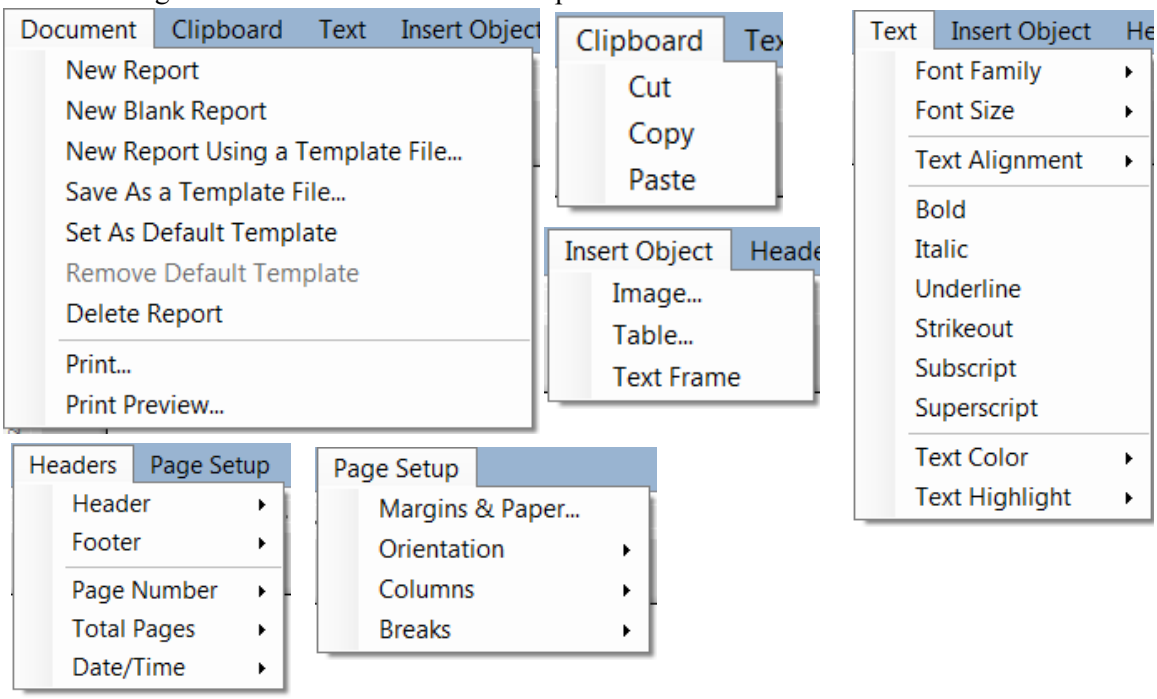


Figure 124

The **Document Tokens** area contains controls for adding selected portions of the available experiment information. A report object can be added by double-clicking the desired item. In this case, the **Experiment Data** group was expanded, then the **Experiment Information** group was expanded, and then

Experiment Information was double-clicked, bringing a table of the contained items over into the Report page on the left:

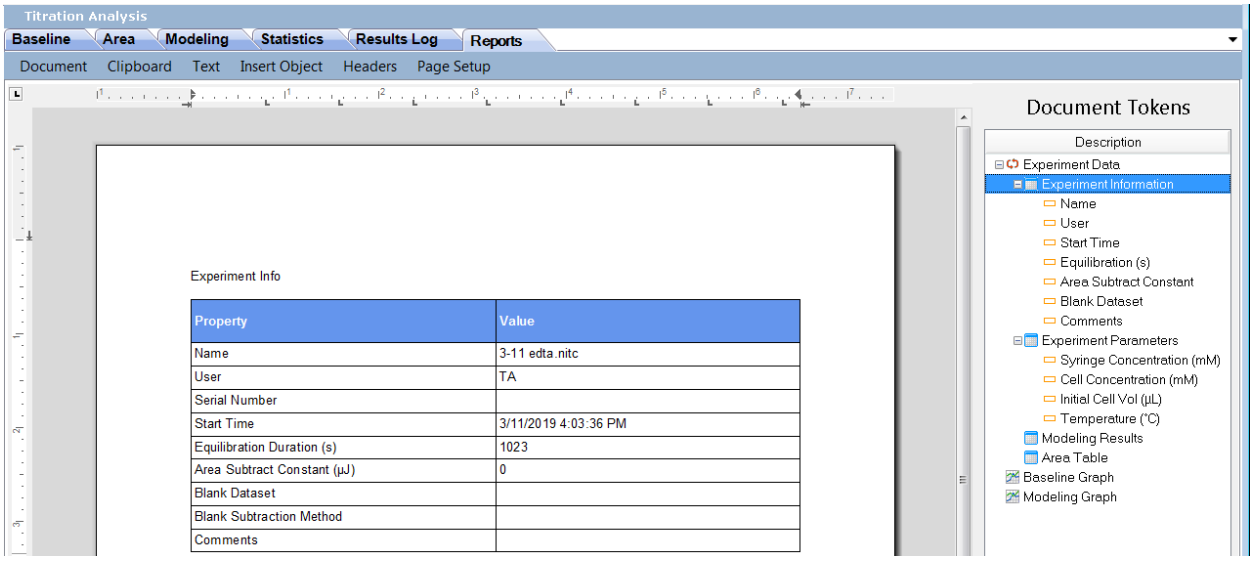


Figure 125

Additional text can be added by clicking at the desired point in the Report and then typing in the content.

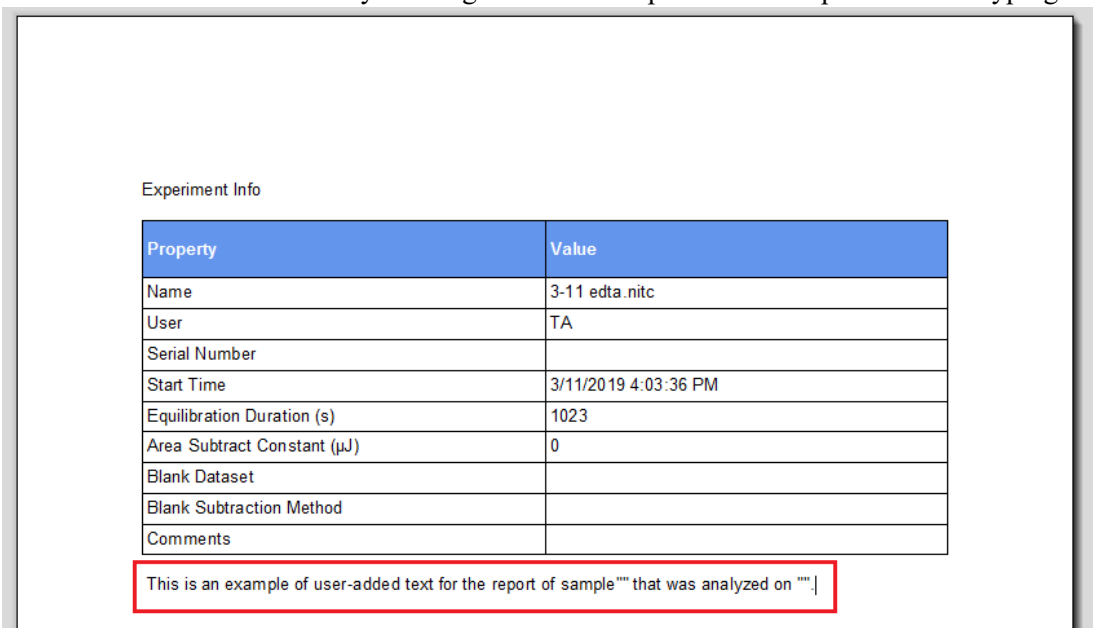


Figure 126

Notice that there was no name or date entered on the example text above. Another feature of the Document Tokens is that sub-items can be inserted as text objects at any point on the report. In the example below the test cursor was first set at the desired insertion point (between the quote marks that had been entered in the

type-in text appearing above), and then the token for the appropriate data object was then double-clicked. This brings the token over to the selected location as new text:

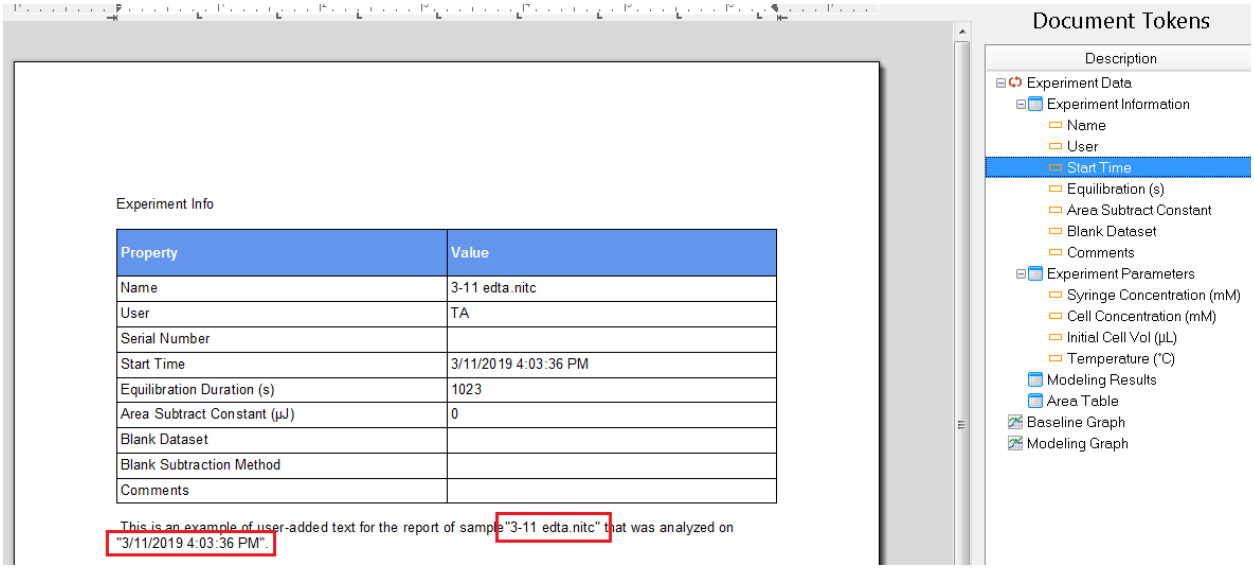


Figure 127

If it is desired to remove any content, select it and press the **Delete** key on the keyboard. Any objects of text can be selected in any of the typical methods for Windows software, such as a single click, a click-and-drag, or click at the start point and then Shift-click at the end point of a section to be deleted.

Continue until the report has been formatted in the desired way. This format can be saved as a Template which can be selected in subsequent work. Another option is to save it as the Default Template, which would then be automatically selected when creating a new report from another experiment.

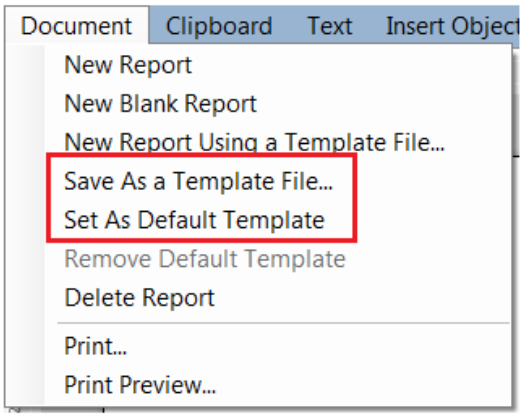


Figure 128

Paper copies of the report may be printed from the **Document** menu. The **Print** dialog also gives an option to create an Adobe PDF format file from the report:

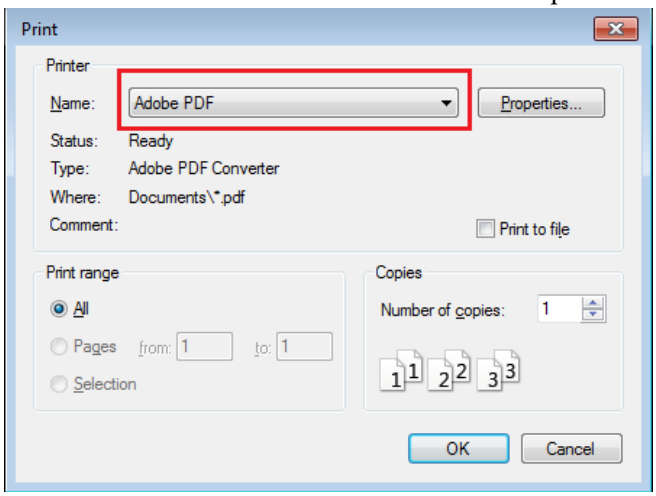


Figure 129

Appendix: B

Processing of Enzyme Kinetics Experiments

NanoAnalyze can process enzyme kinetics experiments using either the Michaelis-Menten or the Lineweaver-Burk methods. To get started, load a kinetics experiment data file into NanoAnalyze. An additional data file from an experiment based on the same chemical system, but with the concentrations adjusted in order to provide simple enthalpy data, can optionally be loaded as well.

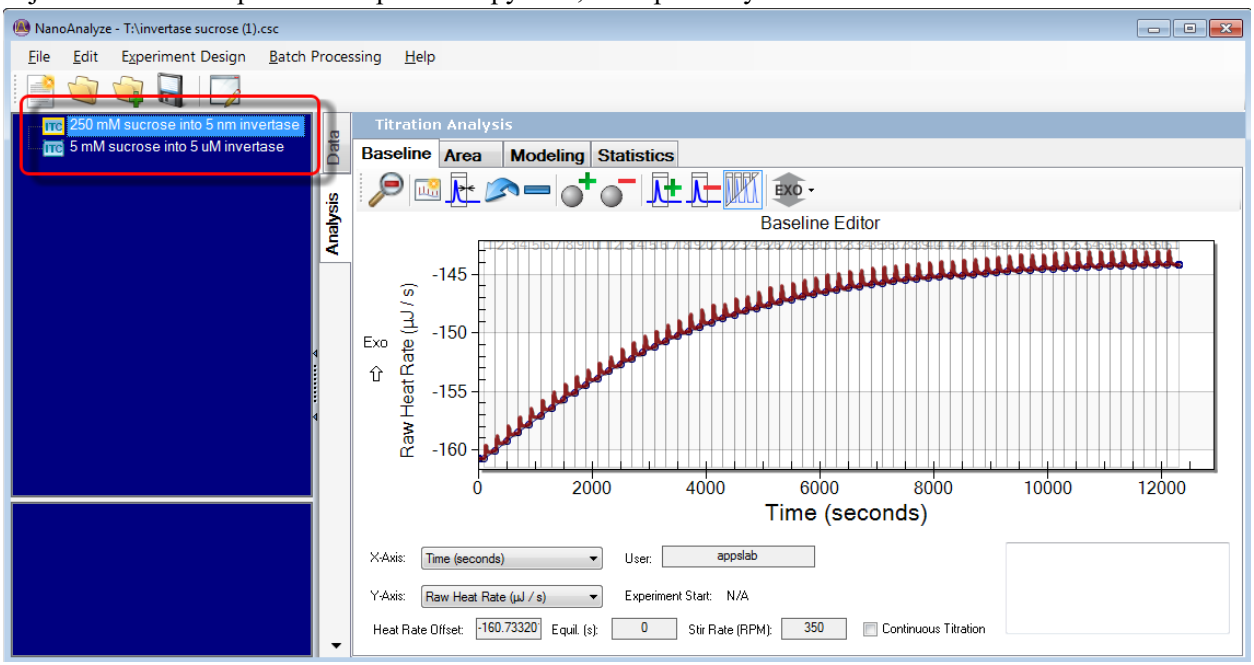


Figure 130

Now the data must be converted into the MIM format. In the File tree on the left side of the NanoAnalyze window right-click the file and select Properties.

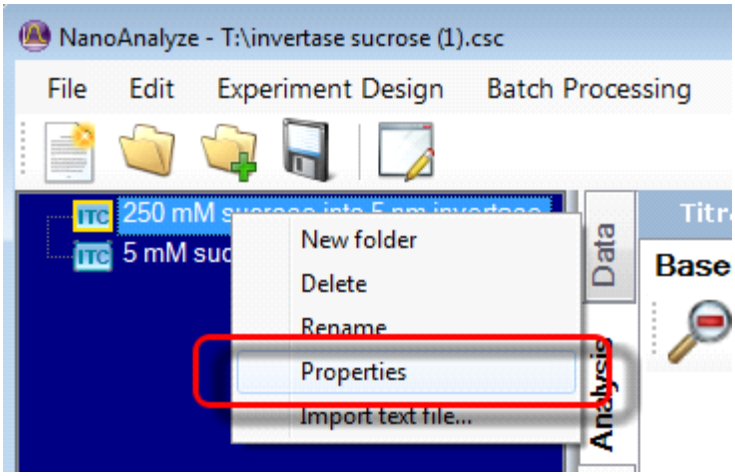


Figure 131

Select "Convert to MIM Enzyme Kinetics" and then click "Ok".

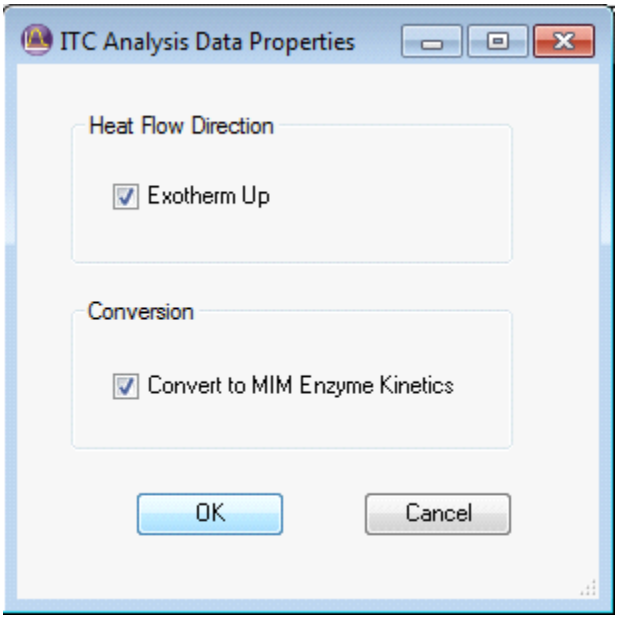


Figure 132

NanoAnalyze is now in the enzyme kinetics analysis mode. New controls and tabs for the processing steps appear above the data window. The concentration values, if they were input in ITCRun software at the time of setting up the experiment, will be carried over into NanoAnalyze. If desired, the units of concentration can be changed in NanoAnalyze software using the drop-down menu at the upper left of this page.

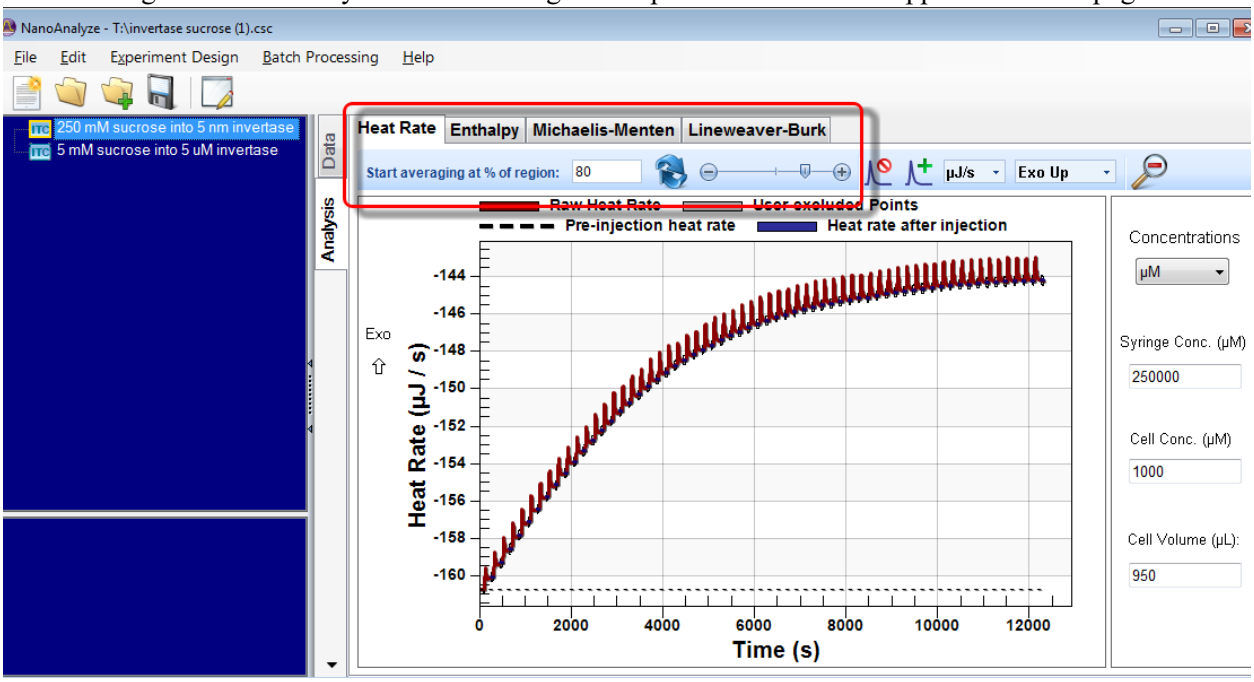


Figure 133

This zoomed-in view of the first several injections shows that analysis windows for each injection interval have been defined at the default range of the final 20% of each injection interval window.

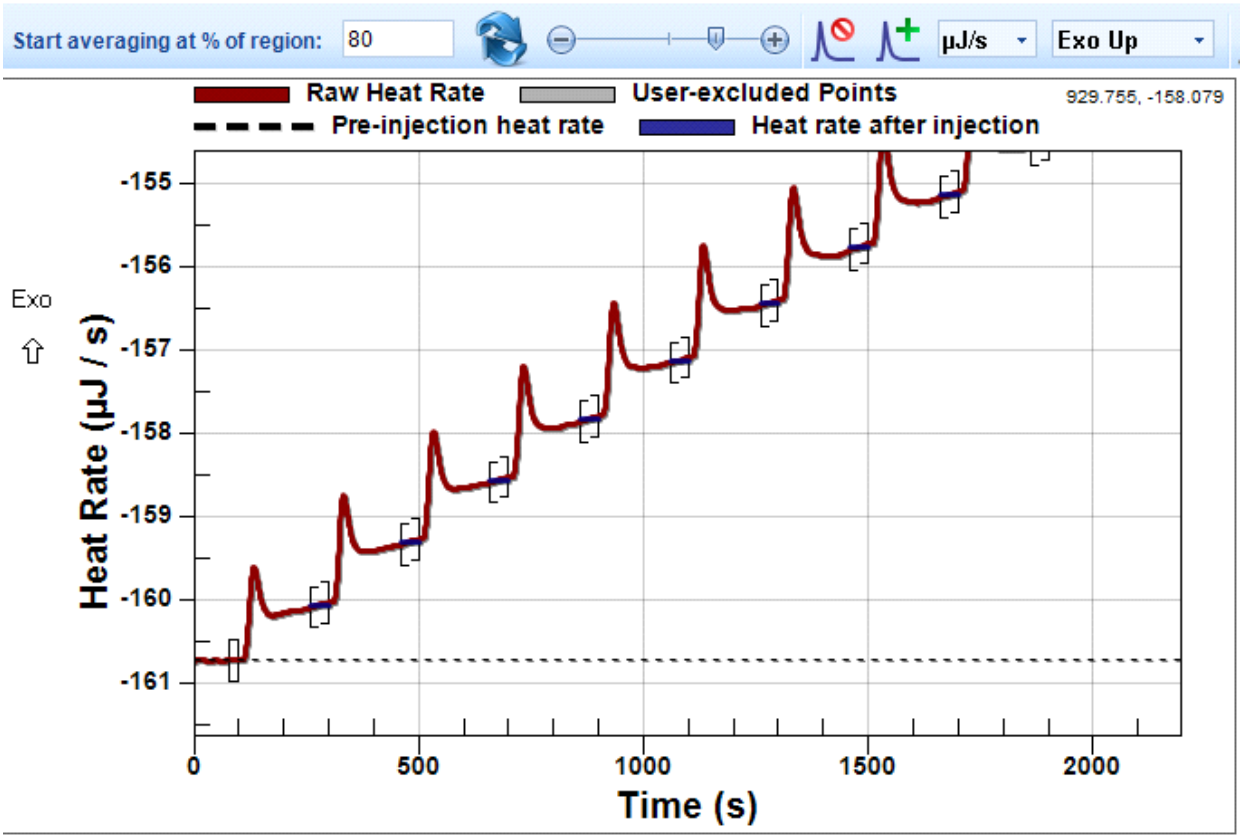


Figure 134

The widths may be adjusted if necessary, by altering the starting times for the windows. The start time can be altered by either entering a percentage figure in the text entry box (click the blue arrow icon to accept the updated number), moving the slider control, or clicking on the "-" or "+" buttons. The ends of the active

data windows are always located at the ends of their injection intervals; only the starting times are adjusted.

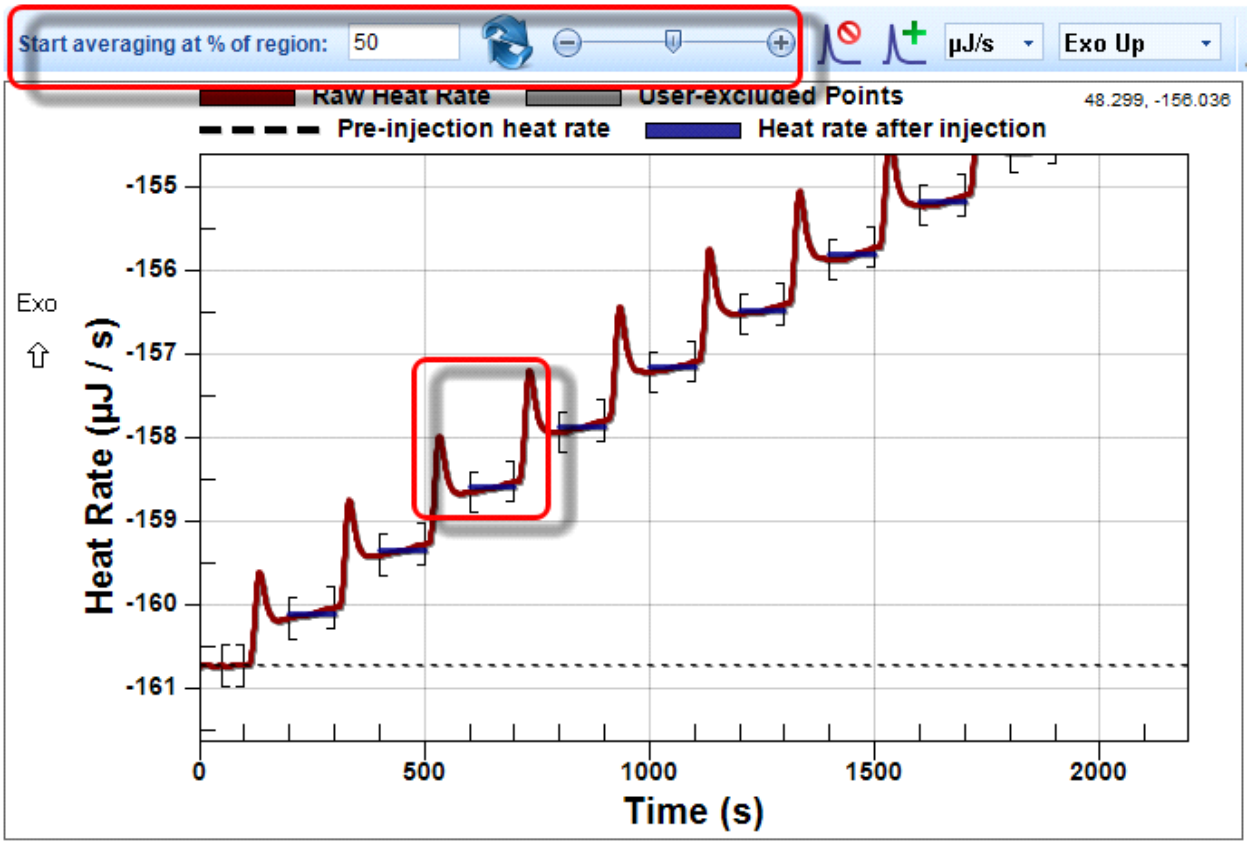


Figure 135

Proceed to the Enthalpy tab. The next step is to enter an enthalpy value for the reaction.

The value can be entered manually if this value is already known. Type the value into the entry window and click the "Apply" button. The manual entry box is highlighted in red.

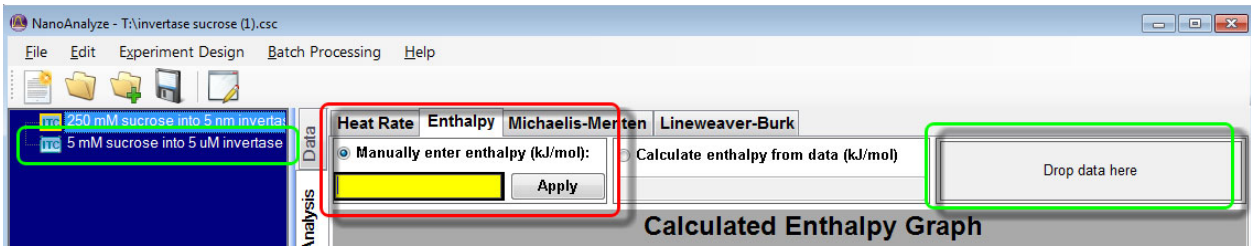


Figure 136

Another method of entering the enthalpy value is to extract it directly from an enthalpy experiment file. An enthalpy-only enzyme experiment typically uses a reduced injection amount to allow the reactions for each injection to proceed to completion within a short time interval. In the example shown below the substrate concentration was reduced by a factor of 25 in comparison to the kinetics experiment. The interval needs to be long enough to insure that the signal returns to baseline. For the enthalpy only titration, the substrate concentration is decreased and the enzyme concentration is increase. Under these conditions of low substrate and high enzyme, the enzyme will convert all of the substrate into product.

Add an enthalpy experiment into NanoAnalyze and it will appear in the file tree at the left of the program window. Click and drag the file over to the box titled "Drop Data Here". These fields are highlighted in green in the prior Figure 136. An average enthalpy value is calculated from all integrated peaks. This example exhibits the typical condition of initial peaks that have reduced areas due to the loss of some reactant from the injection syringe that occurs during the thermal stabilization of the samples in the instrument. The initial peak needs to be discarded in order to allow a correct averaging of the remaining peaks.

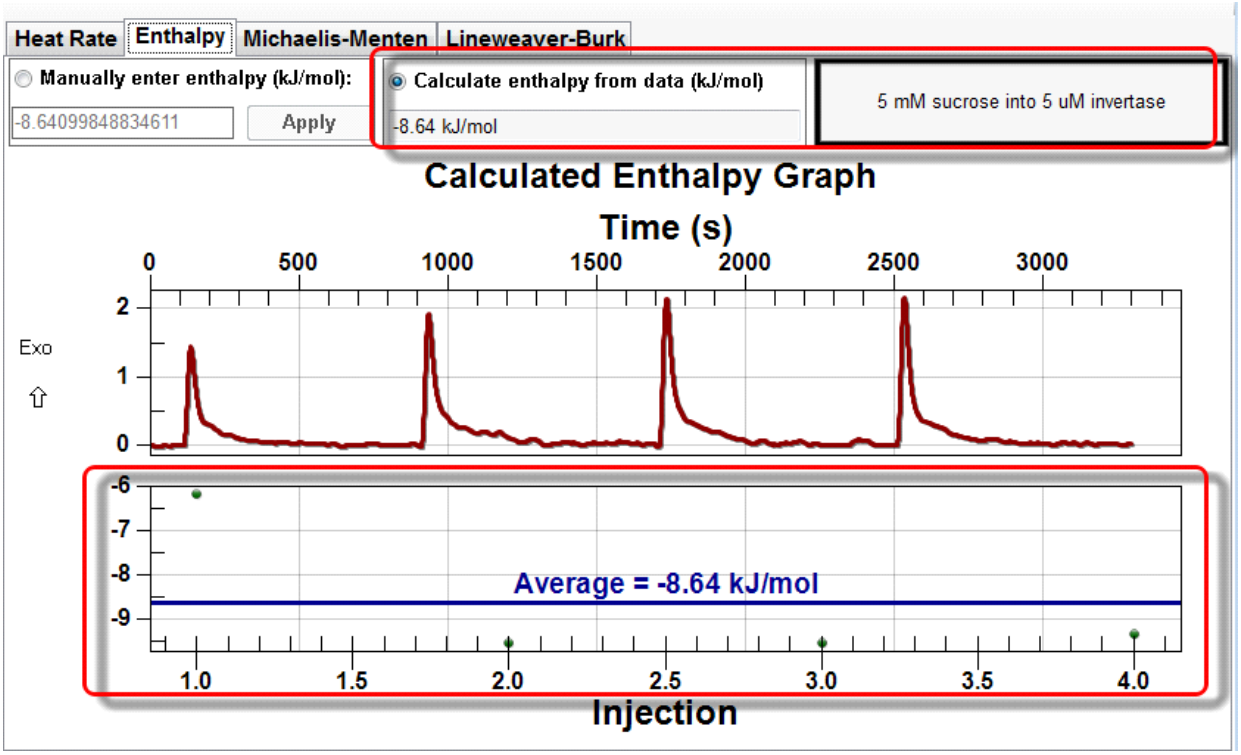


Figure 137

Individual peaks can be deselected by clicking on the appropriate green dot in the lower graph. Deselected dots show as gray instead of green. The average is now calculated on the basis of the remaining injection

peaks, and is now a visibly better representation of the correct average. The value in the calculated enthalpy window is automatically updated.

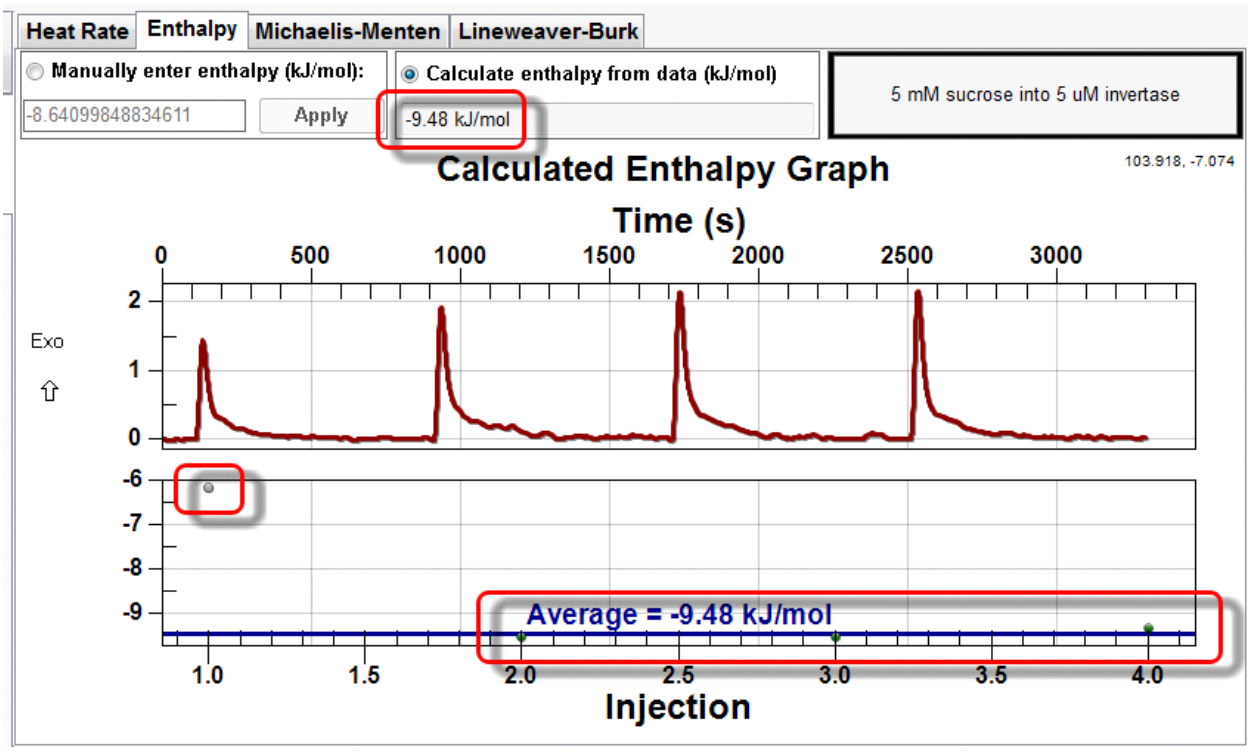


Figure 138

The kinetics calculations are now completed and ready for review. Michaelis-Menten and Lineweaver-Burk analysis methods are presented. Individual data points that might not fit the modeling function, most likely the early injection peaks, can be deselected by clicking on the colored dots. Both analysis methods are updated, and the deselected data points turn gray. There may be a difference between the calculated results of the two methods because of the nonlinear scale transformation, which effectively changes the weighting values of the data points. In the Michaelis-Menten method the weighting is equal for all data points. In the example shown below the data points for the first three injections have been eliminated in

order to allow for an improved fit of the modeling curve. Note that when the view is switched to the Lineweaver-Burk analysis method, the same points are deselected there was well.

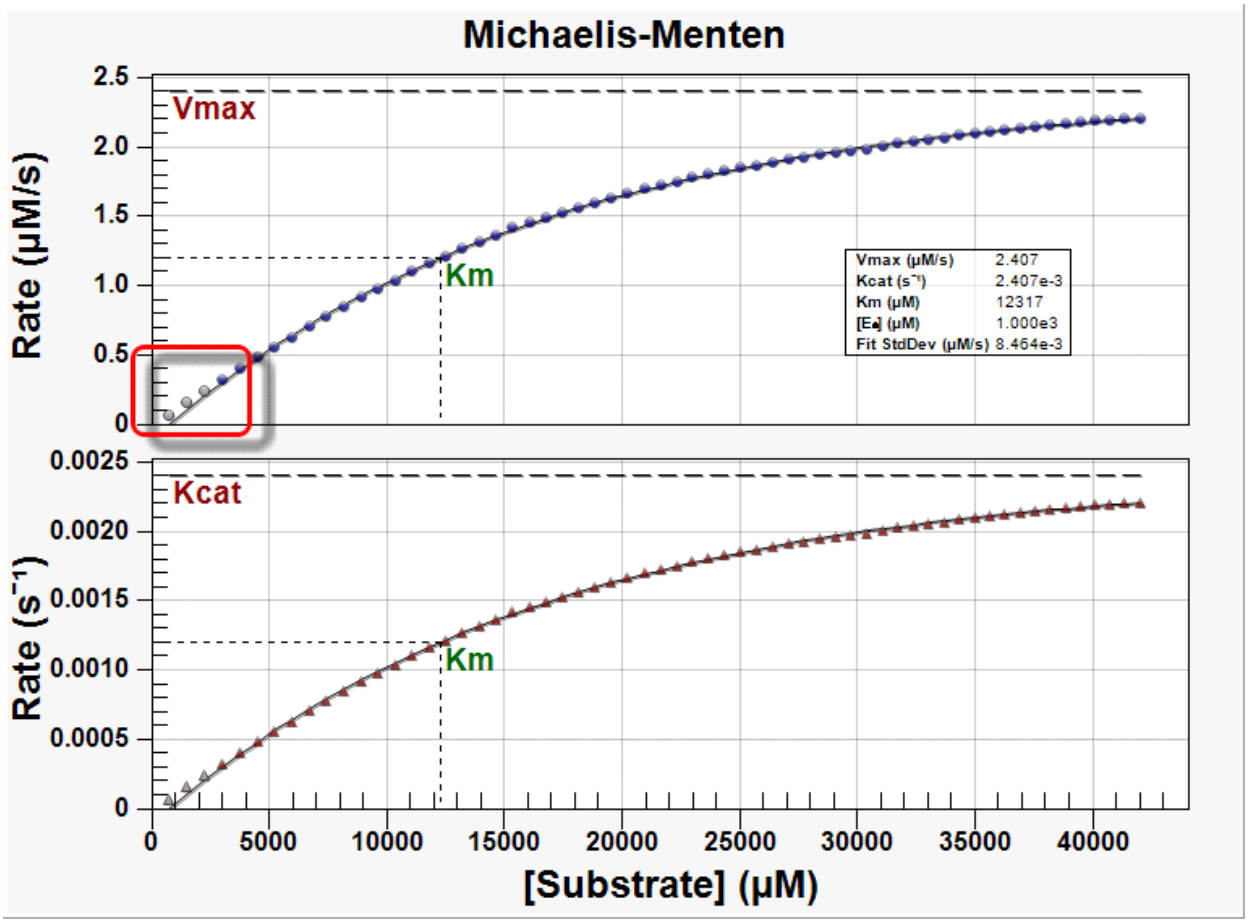


Figure 139

The Lineweaver-Burk method features a stronger weighting of the data points from the later injections, which appear on the right hand side of the chart. Note that any data points which have been eliminated in the Michaelis-Menten analysis tab are also eliminated from the data set used for the Lineweaver-Burk

analysis. Note that because Michaelis-Menten and Lineweaver-Burk have different weightings of the data points in their curve fits, it is possible for the analytical results to differ as well.

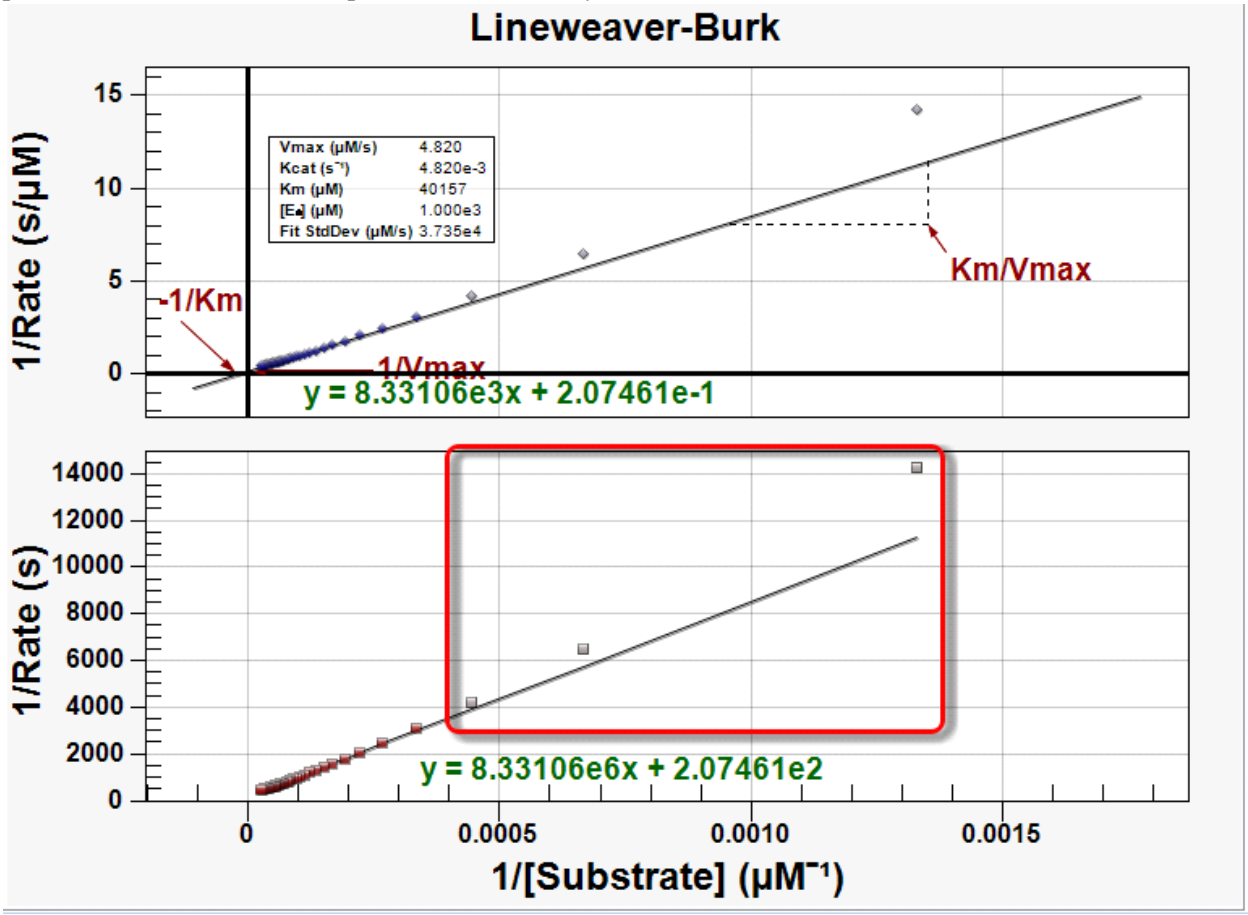


Figure 140

The numeric formats of the data fields in the results table can be adjusted if necessary. Click the Settings icon  to bring up a dialog box for setting the desired numeric formats.

Select the desired number of display digits for any date field and click the "Ok" button when done.

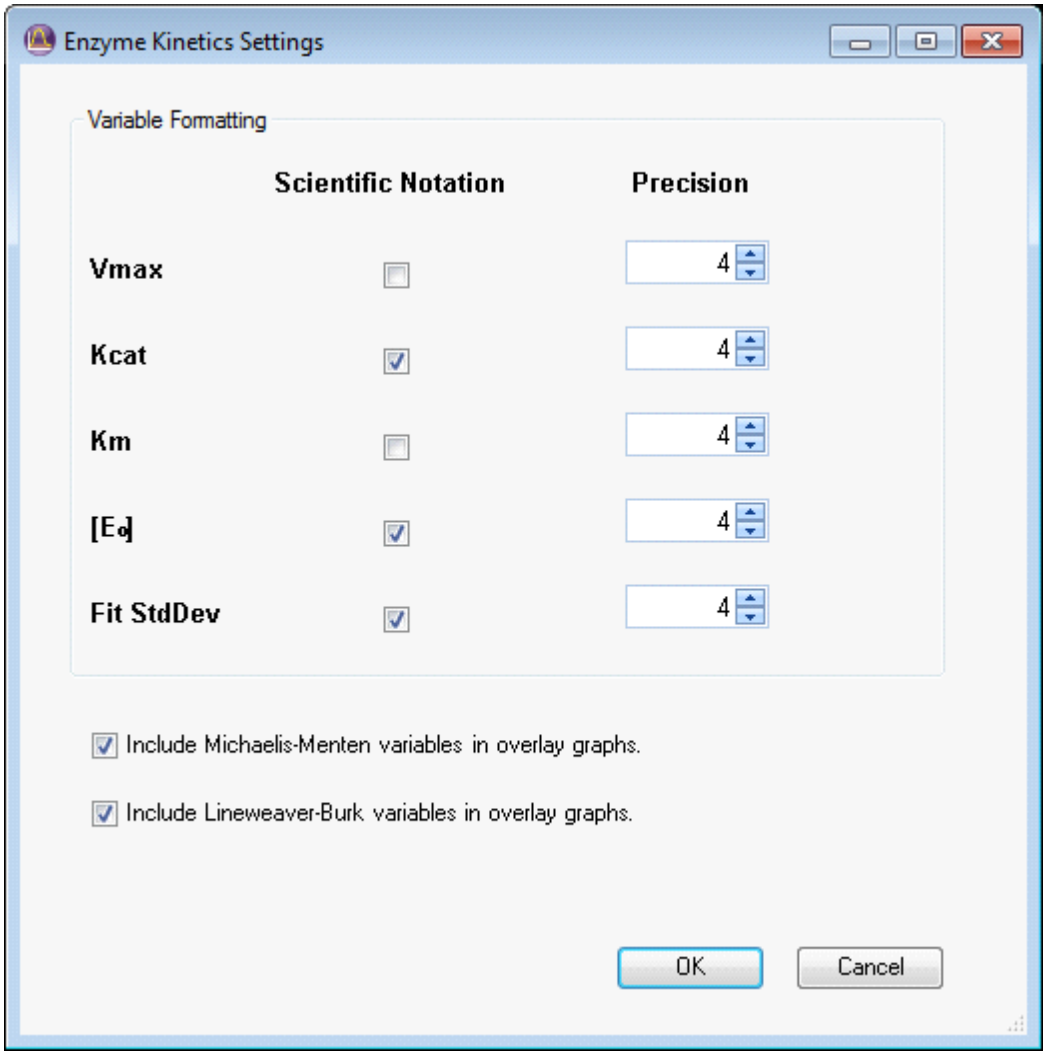


Figure 141

Appendix: C

Models Reference

ITC Binding Model Name: Independent

Main Function

```
' quadratic constants a, b, and c
Dim a As Double =  Ka
Dim b As Double = -Ka * (MolesSyringe(iteration) + MolesCell(iteration) * n) - CellVolume(iteration) / 1e6
Dim c As Double =  Ka * MolesSyringe(iteration) * MolesCell(iteration) * n
Bound = (-b - Sqrt(b*b - 4*a*c)) / (2*a)' Calculate the root using quadratic formula
If OverfillMode Then' Take loss of solution from overfill into account
   OldBound = OldBound * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
Heat = 1e9 * (Bound-Oldbound) * dh' Calculate heat
Oldbound = Bound' Save value of bound for the next iteration of modeldatapoint()
Return Heat
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
OldBound = 0
```

Auxiliary Functions

```
Public Function dS() As Double
  Return (dH * 1000 + R * (Temperature + 273.15) * log(Ka)) / (Temperature + 273.15)
End Function

Public Function Kd() As Double
  Return 1 / Ka
End Function
```

Global Variables and Constants

```
Dim OldBound As Double
Dim OverfillMode As Boolean
Dim Bound As Double
Dim TotalCellVolume As Double
Dim Heat As Double
```

Provided Constants

These constants are automatically created and are available for use in the model.

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Cooperative

Main Function

```
CellVol = CellVolume(iteration) / 1e6
St = (MolesSyringe(iteration) / CellVol)
Ct = (MolesCell(iteration) / CellVol)
XFree = Host.ZBrentRootFinder(0, St, 1e-12, 1000, AddressOf Equation, St, Ct, B1, B2)
Summation = (B1 * XFree * dH1 + B2 * XFree * XFree * dH2) * 1000
    Z = Host.ZBrentPartition(XFree, B1, B2)
Q = 1e6 * Summation * CellVol * Ct / Z
If OverfillMode Then
   PrevQ = PrevQ * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
dQ = Q - PrevQ
PrevQ = Q
Return dQ
```

Initialization Function

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
PrevQ = 0
```

Auxiliary Functions

```
Private Function Equation(ByVal x As Double, ParamArray params() As Double) As Double
    Dim St As Double = params(0)
    Dim Ct As Double = params(1)
    Dim B1 As Double = params(2)
    Dim B2 As Double = params(3)
    Dim z As Double = B1 * x + B2 * 2 * x * x
    Return St - x - Ct * z / Host.Partition(x, B1, B2)
End Function
```

Global Variables and Constants

```
Dim CellVol
Dim St
Dim Ct
Dim gB1
Dim gB2
Dim XFree
Dim Z ' Partition
Dim Summation
Dim Q, dQ
Dim PrevQ
Dim OverfillMode
Dim TotalCellVolume
```

Provided Constants'

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
```

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Model Name: Blank (constant)

Main Function

Return blank

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

(none)

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Model Name: Blank (linear)

Main Function

Dim XValue As Double = iteration + 1

Return slope * XValue + intercept

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

(none)

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Multiple Sites

Main Function

```
cellVol = CellVolume(iteration) / 1e6
St = MolesSyringe(iteration) / cellVol
Ct = MolesCell(iteration) / cellVol
 ' Cubic polynomial constants
Dim a As Double = Ka1 * Ka2
Dim b As Double = Ka1 + Ka2 + a * (Ct * (n1 + n2) - St)
Dim c As Double = 1.0 + Ct * (n1 * Ka1 + n2 * Ka2) - St * (Ka1 + Ka2)
Dim d As Double = -St
Dim roots As Double() = Host.GetCubicEquationRoots(a, b, c, d)
Xfree = roots(0)
If OverfillMode Then
    OverfillDisplacementRatio = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
    OldQ = OldQ * OverfillDisplacementRatio
End If
 Dim Q As Double = 1e9 * cellVol * Ct * (n1 * dH1 * Ka1 * Xfree / (1.0 + Ka1 * Xfree) + n2 * dH2 * Ka2
* Xfree / (1.0 + Ka2 * Xfree))
Dim dQ As Double = Q - OldQ
```

```
OldQ = Q
Return dQ
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
OldQ = 0.0
```

Auxiliary Functions

```
Public Function dS1() As Double
  Return (dH1 * 1000 + R * (Temperature + 273.15) * log(Ka1)) / (Temperature + 273.15)
End Function

 Public Function dS2() As Double
  Return (dH2 * 1000 + R * (Temperature + 273.15) * log(Ka2)) / (Temperature + 273.15)
 End Function

 Public Function Kd1() As Double
  Return 1 / Ka1
 End Function

 Public Function Kd2() As Double
  Return 1 / Ka2
 End Function
```

Global Variables and Constants

```
Dim OldQ
Dim cellVol
Dim St
Dim Ct
Dim Xfree
Dim OverfillMode
Dim OverfillDisplacementRatio
Dim TotalCellVolume
```

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Binding Model Name: Dimer Dissociation

Main Function

```
CellVol = CellVolume(iteration)/1e6
InjVol = InjectionVolume(iteration)/1e6
Ci = MolesSyringe(iteration) / CellVol

'Calculate total dimer for cell
A = 4.0
B = -4.0 * Ci - Kd
C = Ci * Ci
DimerCell = (-B - Math.Sqrt(B*B -4*A*C)) / (2*A)

If OverfillMode Then
    LastDimerCell = LastDimerCell * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If

Dim Heat As Double = 1e9 * dH *  ( (DimerSyr * InjVol) - ((DimerCell - LastDimerCell) * CellVol) )
LastDimerCell = DimerCell
Return Heat
```

Initialization Functions

```
LastDimerCell = 0

OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
```

```
'calculate total dimer for syringe
Csyr = TitrantmM / 1e3
A = 4
B = -4.0 * Csyr - Kd
C = Csyr * Csyr
DimerSyr = (-B -Math.Sqrt(B*B - 4*A*C)) / (2*A)
```

Auxiliary Functions

```
Public Function dS() As Double
  Return (dH * 1000 + R * (Temperature + 273.15) * log(1.0 / Kd)) / (Temperature + 273.15)
End Function


Public Function Ka() As Double
  Return 1.0 / Kd
End Function
```

Global Variables and Constants

```
Dim CellVol As Double
Dim InjVol As Double
Dim Csyr As Double
Dim Ci As Double
Dim A As Double
Dim B As Double
Dim C As Double
Dim DimerSyr As Double
Dim DimerCell  As Double
Dim Dimer As Double
Dim LastDimerCell As Double
Dim OldDimer As Double
Dim OverfillMode As Boolean
Dim TotalCellVolume As Double
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
```

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

ITC Binding Model Name: Competitive Replacement

Main Function

totalL2 = MolesSyringe(iteration) / V

totalP = MolesCell(iteration) / V

totalL1 = totalP * (L1mM / TitratemM)

' Displacement calculation (Experiment must be run in "overfill" mode.)

OverfillDisplacementRatio = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume

LastPL1 = LastPL1 * OverfillDisplacementRatio

LastPL2 = LastPL2 * OverfillDisplacementRatio

PL1 = Host.Minimize(PL1_Estimate, 1.7e-308, max, 2, AddressOf PL1Minimization, Ka1, Ka2, totalP, totalL1, totalL2)

PL2 = (totalP - (PL1/((totalL1-PL1)*Ka1)) - PL1)

max = PL1 ' PL1 decreases with each injection

PL1_Estimate = PL1 ' Next estimate

Dim Heat As Double

If iteration = 0 Then

    Heat = 0.0

Else

   Heat = (((PL1-LastPL1) * dH1)+((PL2-LastPL2) * dH2)) * V * 1e9

End If

LastPL1 = PL1

LastPL2 = PL2

Return Heat

Initialization Functions

TotalCellVolume = CellVolume(0)

```
LastPL1 = 0
LastPL2 = 0
V = CellVolume(0) / 1000000.0
totalP = MolesCell(0) / V
max = totalP
PL1_Estimate = GetPL1Estimate(1.7e-308, max, Ka1, Ka2, totalP, totalP * (L1mM / TitratemM), MolesSyringe(0) / V)
```

Auxiliary Functions

```
Public Function dS1() As Double
  Return (dH1 * 1000 + R * (Temperature + 273.15) * log(Ka1)) / (Temperature + 273.15)
End Function

Public Function dS2() As Double
  Return (dH2 * 1000 + R * (Temperature + 273.15) * log(Ka2)) / (Temperature + 273.15)
End Function

Public Function Kd1() As Double
  Return 1 / Ka1
End Function

Public Function Kd2() As Double
  Return 1 / Ka2
End Function

Public Function PL1Minimization(ByVal parameters() As Double) As Double
  Dim PL1 As Double = parameters(0) ' The first parameter is the independent variable
  Dim Ka1 As Double = parameters(1)
  Dim Ka2 As Double = parameters(2)
  Dim totalP As Double = parameters(3)
  Dim totalL1 As Double = parameters(4)
  Dim totalL2 As Double = parameters(5)
  Dim EquationLeftSide As Double
  Dim EquationRightSide As Double
  EquationLeftSide = Ka1 * ((totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1) * (totalL1-PL1))
```

```
  EquationRightSide = Ka2 * (totalL2 - (totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1)) * PL1
  Return EquationLeftSide - EquationRightSide
End Function


Private Function GetPL1Estimate(ByVal min As Double, ByVal max As Double, ByVal Ka1 As Double,
ByVal Ka2 As Double,  ByVal totalP As Double, ByVal totalL1 As Double, ByVal totalL2 As Double) As
Double
  Dim EquationLeftSide As Double
  Dim EquationRightSide As Double
  Dim MinDifference As Double = Double.MaxValue
  Dim Difference As Double
  Dim PL1 As Double
  Dim i As Integer
  Dim MinDifferenceIndex As Integer
  For i = 0 To 100
    PL1 = min + i * (max - min)  / 100.0
    EquationLeftSide = Ka1 * ((totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1) * (totalL1-PL1))
    EquationRightSide = Ka2 * (totalL2 - (totalP - (PL1 / ((totalL1-PL1) * Ka1)) - PL1)) * PL1
    Difference = Math.Abs(EquationLeftSide - EquationRightSide)
    If (Difference < MinDifference)  Then
      MinDifference = Difference
      MinDifferenceIndex = i
    End If
  Next i
  Return min + MinDifferenceIndex * (max - min)  / 100.0
End Function
```

Global Variables and Constants

```
 Dim PL1 As Double
 Dim PL2 As Double
 Dim LastPL1 As Double
 Dim LastPL2 As Double
 Dim PL1_Estimate As Double
 Dim totalL1 As Double
 Dim totalL2 As Double
 Dim totalP As Double
```

```
Dim max As Double
Dim V As Double
Dim TotalCellVolume As Double
Dim OverfillDisplacementRatio As Double
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Cooperative

Main Function

```
Dim xf
Dim z
gB1 = b1
gB2 = b2
CellVol = CellVolume(iteration) / 1e6
St = (MolesSyringe(iteration) / CellVol)
Ct = (MolesCell(iteration) / CellVol)
xf = Host.ZBrent(0, St, 1e-12, gB1, gB2, St, Ct)
z = (b1 * xf * dH1 + b2 * xf * xf * dH2) * 1000
heat = 1e6 * z * CellVol * Ct / Host.ZBrentPartition(xf, gB1, gB2)
If OverfillMode Then
  b = b + heat * (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
End If
heat = heat + b
Dim deltaHeat As Double = heat - heatOld
heatOld = heat
Return deltaHeat
```

Initialization Functions

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
heatOld = 0
b = 0
```

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim CellVol
Dim St
Dim Ct
Dim gB1
Dim gB2
Dim heat
Dim heatOld
Dim b
Dim OverfillMode
Dim TotalCellVolume
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Enthalpy Screening

Main Function

```
Return Qmean()
```

Initialization Function

```
count = CorrectedQ.Length
GetInjectedMoles()
CalcStats()
CalcChemistry()
```

Auxiliary Functions

```
Public Function Qmean() As Double
    Return _Qmean
End Function

Public Function QStdDev() As Double
    Return _QStdDev
End Function

Public Function dH() As Double
    Return _dH
End Function

Public Function dG() As Double
    Return _dG
End Function

Public Function minusTdS() As Double
    Return _minusTDeltaS
End Function

Public Function dHse() As Double
    Return _dHse
```

```
End Function

Private Sub CalcStats()

  Dim index As Integer
  Dim sum As Double = 0
  Dim actualCount As Integer = 0

 ' Calculate mean
For index = 0 To count - 1
      If Not (Host.IsPointExcluded(index)) Then
   sum = sum + CorrectedQ(index)
            actualCount = actualCount + 1
           End If
Next index

If (actualCount = 0) Then
         _Qmean = 0.0
         _QStdDev = 0.0
         Exit Sub
 Else
         _Qmean = sum / actualCount
 End If

 ' Calculate Standard Deviation
sum = 0
For index = 0 To count - 1
       If Not (Host.IsPointExcluded(index)) Then
                sum = sum + (CorrectedQ(index) - Qmean) * (CorrectedQ(index) - Qmean)
                  actualCount = actualCount + 1
                End If
Next index

_QStdDev = Math.Sqrt(sum / count)
```

```
End Sub

Private Sub CalcChemistry()

  Dim index As Integer
  Dim sum As Double = 0
  Dim actualCount As Integer = 0

' Calculate dH
For index = 0 To count - 1
          If Not (Host.IsPointExcluded(index)) Then
   sum = sum + CorrectedQ(index) / (InjectedMoles(index) * 1e9) ' Convert to kJ/mol
            actualCount = actualCount + 1
            End If
Next index

If (actualCount = 0) Then
     _dH = 0.0
     _dG = 0.0
     _minusTDeltaS = 0.0
     _dHse = 0.0
     Exit Sub
Else
     _dH = sum / actualCount
End If

_dG = -R * (Temperature + 273.15) * Math.Log(1 / (Kd * 1e-6)) / 1000
_minusTDeltaS = _dG - _dH

' Calculate Standard Error for dH
sum = 0
For index = 0 To count - 1
          If Not (Host.IsPointExcluded(index)) Then
                         Dim Peak_dH As Double = CorrectedQ(index) / (InjectedMoles(index) * 1e9)
                sum = sum + (Peak_dH - _dH) * (Peak_dH - _dH)
```

```
                actualCount = actualCount + 1
            End If
        Next index

        Dim dH_StdDev As Double = Math.Sqrt(sum / count)
        _dHse = dH_StdDev / Math.Sqrt(count)

    End Sub

    Private Sub GetInjectedMoles()

        Dim index As Integer
        ReDim InjectedMoles(count)

        For index = count - 1 To 0 Step -1
            InjectedMoles(index) = InjectionVolume(index) * TitrantmM * 1e-9
    Next index

    End Sub
```

Global Variables and Constants

```
Dim count As Integer
Dim _Qmean, _QStdDev As Double
Dim _dH, _dHse, _dG, _minusTDeltaS As Double
Dim InjectedMoles() As Double
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Sequential Three Site

Main Function

```
cellVol = CellVolume(iteration) / 1e6
St = (MolesSyringe(iteration) / CellVol)
 Ct = MolesCell(iteration) / cellVol

_Ka1 = 1 / Kd1
_Ka2 = 1 / Kd2
_Ka3 = 1 / Kd3
Dim B1 As Double = _Ka1
Dim B2 As Double = _Ka1 * _Ka2
Dim B3 As Double = _Ka1 * _Ka2 * _Ka3

If OverfillMode Then
        ' Take loss of solution from overfill into account
         Dim OverfillFactor = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
         PreviousQ = PreviousQ * OverfillFactor
         PreviousXFree = PreviousXFree * OverfillFactor
 End If

' Calculate the root using ZBrentalgorithm
  XFree = Host.ZBrentRootFinder(PreviousXFree, St, 1e-16, 1000, AddressOf Equation, St, Ct, B1,
B2, B3)

Dim P As Double = Host.Partition(XFree, B1, B2, B3)
Dim F1 As Double = B1 * XFree / P
Dim F2 As Double = B2 * XFree * XFree / P
Dim F3 As Double = B3 * XFree * XFree * XFree / P

' Calculate heat
  Q = 1e9 * cellVol * Ct * (F1 * dH1 + F2 * (dH1 + dH2) + F3 * (dH1 + dH2 + dH3))
dQ = Q - PreviousQ

' Save value of heat for the next iteration
```

```
 PreviousQ = Q
PreviousXFree = XFree

Return dQ
```

Initialization Function

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
PreviousQ = 0
PreviousXFree = 0
```

Auxiliary Functions

```
Public Function Ka1() As Double
        Return 1 / Kd1
End Function

Public Function Ka2() As Double
        Return 1 / Kd2
End Function

Public Function Ka3() As Double
        Return 1 / Kd3
End Function

Public Function dS1() As Double
    Return (dH1 * 1000 + R * (Temperature + 273.15) * log(1/Kd1)) / (Temperature + 273.15)
End Function

Public Function dS2() As Double
   Return (dH2 * 1000 + R * (Temperature + 273.15) * log(1/Kd2)) / (Temperature + 273.15)
End Function

Public Function dS3() As Double
   Return (dH3 * 1000 + R * (Temperature + 273.15) * log(1/Kd3)) / (Temperature + 273.15)
```

```
End Function

Private Function Equation(ByVal x As Double, ParamArray params() As Double) As Double
        Dim St As Double = params(0)
           Dim Ct As Double = params(1)
           Dim B1 As Double = params(2)
           Dim B2 As Double = params(3)
           Dim B3 As Double = params(4)
                   Dim SumOf_iFi As Double = B1 * x + B2 * 2 * x * x + B3 * 3 * x * x * x
          Dim DegreeOfSaturation = SumOf_iFi / Host.Partition(x, B1, B2, B3)
   Return St - x - Ct * DegreeOfSaturation
End Function
```

Global Variables and Constants

```
Dim cellVol As Double
Dim St, Ct As Double
Dim Q, dQ, PreviousQ As Double
Dim XFree, PreviousXFree As Double
Dim TotalCellVolume As Double
Dim _Ka1, _Ka2, _Ka3 As Double
Dim OverfillMode As Boolean
```

Provided Constants

```
Private Readonly TitrantmM as Double = 0.1
Private Readonly TitratemM as Double = 1
Private Readonly InitTitrateVol as Double = 950
Private Readonly DefaultInjVol as Double = 5
Private Readonly Temperature as Double = 25
Private Readonly UsingMicroCalorieUnits as Boolean = False
Private Readonly R as Double = 8.3144621
```

ITC Binding Model Name: Sequential Two Site

Main Function

```
cellVol = CellVolume(iteration) / 1e6
St = (MolesSyringe(iteration) / CellVol)
 Ct = MolesCell(iteration) / cellVol

_Ka1 = 1 / Kd1
_Ka2 = 1 / Kd2

Dim B1 As Double = _Ka1
Dim B2 As Double = _Ka1 * _Ka2

If OverfillMode Then
        ' Take loss of solution from overfill into account
        Dim OverfillFactor = (TotalCellVolume - InjectionVolume(iteration)) / TotalCellVolume
         PreviousQ = PreviousQ * OverfillFactor
        PreviousXFree = PreviousXFree * OverfillFactor
  End If

' Calculate the root using ZBrentalgorithm
  XFree = Host.ZBrentRootFinder(PreviousXFree, St, 1e-16, 1000, AddressOf Equation, St, Ct, B1,
B2)

Dim P As Double = Host.Partition(XFree, B1, B2)
Dim F1 As Double = B1 * XFree / P
Dim F2 As Double = B2 * XFree * XFree / P

' Calculate heat
  Q = 1e9 * cellVol * Ct * (F1 * dH1 + F2 * (dH1 + dH2))
dQ = Q - PreviousQ

' Save value of heat for the next iteration
 PreviousQ = Q
PreviousXFree = XFree
```

```
Return dQ
```

Initialization Function

```
OverfillMode = (CellVolume(1) = CellVolume(0))
TotalCellVolume = CellVolume(0)
PreviousQ = 0
PreviousXFree = 0
```

Auxiliary Functions

```
Public Function Ka1() As Double
        Return 1 / Kd1
End Function

Public Function Ka2() As Double
        Return 1 / Kd2
End Function

Public Function dS1() As Double
   Return (dH1 * 1000 + R * (Temperature + 273.15) * log(1/Kd1)) / (Temperature + 273.15)
End Function

Public Function dS2() As Double
   Return (dH2 * 1000 + R * (Temperature + 273.15) * log(1/Kd2)) / (Temperature + 273.15)
End Function

Private Function Equation(ByVal x As Double, ParamArray params() As Double) As Double
        Dim St As Double = params(0)
        Dim Ct As Double = params(1)
        Dim B1 As Double = params(2)
        Dim B2 As Double = params(3)
        Dim SumOf_iFi As Double = B1 * x + B2 * 2 * x * x
        Dim DegreeOfSaturation = SumOf_iFi / Host.Partition(x, B1, B2)
        Return St - x - Ct * DegreeOfSaturation
```

End Function

Global Variables and Constants

Dim cellVol As Double

Dim St, Ct As Double

Dim Q, PreviousQ, dQ As Double

Dim XFree, PreviousXFree As Double

Dim TotalCellVolume As Double

Dim _Ka1, _Ka2 As Double

Dim OverfillMode As Boolean

Provided Constants

Private Readonly TitrantmM as Double = 0.1

Private Readonly TitratemM as Double = 1

Private Readonly InitTitrateVol as Double = 950

Private Readonly DefaultInjVol as Double = 5

Private Readonly Temperature as Double = 25

Private Readonly UsingMicroCalorieUnits as Boolean = False

Private Readonly R as Double = 8.3144621

DSC Model Name: Two State

Main Function

```
Dim Y As Double


T = Temp(iteration) + 273.15
Tm0 = Tm + 273.15
dH0 = dH * 1000.0
K=exp(-dH0*(1-T/Tm0)/(R*T))
Cp=(dH0 * dH0)/(R*T*T)*exp(dH0*(-Tm0+T)/(Tm0*T*R))/((1+K)*(1+K))
Y = Cp / 1000.0
Return Y
```

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim T As Double
Dim Tm0 As Double
Dim dH0 As Double
Dim K As Double
Dim Cp As Double
```

Provided Constants

```
Private Readonly Volume as Double = 0.3
Private Readonly Mass as Double = 1
Private Readonly Conc as Double = 0.1
Private Readonly MW as Double = 10
Private Readonly PSV as Double = 0.73
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
```

Private Readonly c as Double = -0.4807

Private Readonly R as Double = 8.3144621

DSC Model Name: Two State Scaled

Main Function

```
Dim Y As Double


T = Temp(iteration) + 273.15
Tm0 = Tm + 273.15
dH0 = dH * 1000.0
K=exp(-dH0*(1-T/Tm0)/(R*T))
Cp=(dH0 * dH0)/(R*T*T)*exp(dH0*(-Tm0+T)/(Tm0*T*R))/((1+K)*(1+K)) * Aw
Y = Cp / 1000.0


Return Y
```

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim T As Double
Dim Tm0 As Double
Dim dH0 As Double
Dim K As Double
Dim Cp As Double
```

Provided Constants

Private Readonly Volume as Double = 0.3

Private Readonly Mass as Double = 1

Private Readonly Conc as Double = 0.1

Private Readonly MW as Double = 10

```
Private Readonly PSV as Double = 0.73
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
Private Readonly c as Double = -0.4807
Private Readonly R as Double = 8.3144621
```

DSC Model Name: General

Main Function

```
Dim Y As Double
bbCp = b
ccCp = c
Tm0 = Tm + 273.15
dHTm = dH * 1000
dCpTm = dCp * 1000
dSTm = dHTm / Tm0
acon = dCpTm - bbCp * Tm0 - ccCp * (Tm0 * Tm0)
T = Temp(iteration) + 273.15
dCp1 = acon + bbCp*T + ccCp*T*T
deltah = dHTm+acon*(T-Tm0)+(bbCp*(T*T-Tm0*Tm0)/2)+(ccCp*(T*T*T-Tm0*Tm0*Tm0)/3)
deltas = dSTm+acon*log(T/Tm0)+bbCp*(T-Tm0)+(ccCp*(T*T-Tm0*Tm0)/2)
kEQ = Exp(-(deltah-T*deltas)/(R*T))
qpar = 1 + kEQ
advh = kEQ * deltah
advh2 = kEQ * deltah * deltah
advcp = kEQ * dCp1
Temp0 = Temp(iteration) + 273.15
dCp1 = acon + bbCp*Temp0 + ccCp*Temp0*Temp0
deltah = dHTm+acon*(Temp0-Tm0)+(bbCp*(Temp0*Temp0-Tm0*Tm0)/
2)+(ccCp*(Temp0*Temp0*Temp0-Tm0*Tm0*Tm0)/3)
deltas = dSTm+acon*log(Temp0/Tm0)+bbCp*(Temp0-Tm0)+(ccCp*(Temp0*Temp0-Tm0*Tm0)/2)
kEQ = Math.Exp(-(deltah-Temp0*deltas)/(R*Temp0))
qpar = 1 + kEQ
advh = kEQ * deltah
advh2 = kEQ * deltah * deltah
```

```
advcp = kEQ * dCp1
hav1 = (advh*advh)/(qpar*qpar)
hav2 = advh2 / qpar
Cp = (advcp/qpar) + (hav2-hav1)/(R*Temp0*Temp0)+(A0 + A1 * Temp(iteration)) * MW * 1000
Y = Cp / 1000
Return Y
```

Initialization Functions

(none)

Auxiliary Functions

(none)

Global Variables and Constants

```
Dim acon As Double
Dim T As Double
Dim deltah As Double
Dim deltas As Double
Dim kEQ As Double
Dim qpar As Double
Dim advh As Double
Dim advh2 As Double
Dim advcp As Double
Dim Temp0 As Double
Dim dCp1 As Double
Dim hav1 As Double
Dim hav2 As Double
Dim Cp As Double
```

Provided Constants

```
Private Readonly Volume as Double = 0.3
Private Readonly Mass as Double = 1
Private Readonly Conc as Double = 0.1
Private Readonly MW as Double = 10
Private Readonly PSV as Double = 0.73
```

```
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
Private Readonly c as Double = -0.4807
Private Readonly R as Double = 8.3144621
```

DSC Model Name: Gaussian

Main Function

```
Dim Y As Double
T = Temp(iteration)
Y = a1*exp(-((T-b1)/c1)^2)
Return Y
```

Initialization Functions

(none)

Auxiliary Functions

```
Public Function area() As Double
  Return a1*c1*1.772453851 'number is for sqr(PI)
End Function
```

Global Variables and Constants

```
Dim T As Double
```

Provided Constants

```
Private Readonly Volume as Double = 0.3
Private Readonly Mass as Double = 1
Private Readonly Conc as Double = 0.1
Private Readonly MW as Double = 10
Private Readonly PSV as Double = 0.73
Private Readonly CalUnits as Boolean = False
Private Readonly b as Double = 300.96
Private Readonly c as Double = -0.4807
Private Readonly R as Double = 8.3144621
```

DSC Model Name: Cooperative

Main Function

```
Dim Y As Double

T = Temp(iteration) + 273.15
Tm0 = Tm + 273.15
dH0 = dH * 1000.0
        dH0cal = Calorimetric_dH * 1000.0
Ka = exp(-dH0cal * (1.0 - T / Tm0) / (R * T))
Cp = Ka * dH0 * dH0cal / ((1.0 + Ka) * (1.0 + Ka) * R * T * T)
Y = Cp / 1000.0

Return Y
```

Initialization Function

(none)

Auxiliary Function

(none)

Global Variables and Constants

```
Dim T As Double
Dim Tm0 As Double
Dim dH0 As Double
Dim dH0cal As Double
Dim Ka As Double
Dim Cp As Double
```

Provided Constants

Private Readonly Volume as Double = 0.3

Private Readonly Mass as Double = 1

Private Readonly Conc as Double = 0.1

Private Readonly MW as Double = 10

Private Readonly PSV as Double = 0.73

Private Readonly CalUnits as Boolean = False

Private Readonly b as Double = 300.96

Private Readonly c as Double = -0.4807

Private Readonly R as Double = 8.3144621

Private Readonly Calorimetric_dH as Double = 0