

# AN.Stability Analysis

USER MANUAL

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NanoTemper® Technologies' Andromeda series instruments detect changes in the fluorescence of GFP fusion and labelled His-tagged proteins over a wide range of temperatures. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures.

The AN.Stability Analysis software is dedicated to analyzing thermal unfolding data acquired with AN.Control software on Andromeda and Andromeda Plex instruments. Combine any data you generated to quickly pinpoint trends and provide statistical relevance from your merged replicate data sets.

## 1. Technical Information

### 1.1. System Requirements

If the necessary licenses have been purchased, AN.Stability Analysis software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

<b>Operating system:</b>	Windows 7 64 Bit or higher
<b>CPU:</b>	Intel Core i5 or better
<b>RAM:</b>	8 GB or more
<b>Hard disk:</b>	20 GB or more free disk space available
<b>Display resolution:</b>	1600 x 900 or better
<b>Software:</b>	Microsoft .NET 4.6.2 framework (included in installer of AN.Stability Analysis software)
<b>Operating system language:</b>	English or German

An external computer mouse is necessary to access all software features.

## 1.2. Compatibility

Measurements collected with all versions of AN.Control are compatible with AN.Stability Analysis.

## 1.3. Installation and License Activation

The software can be installed on any computer and will automatically start in trial mode, when opened for the first time. The trial version offers full functionality and has to be activated with a license key purchased from NanoTemper Technologies after 30 days.

To activate the license, navigate to the main menu and select *Licensing Status*. Please follow the instructions provided. One software license is valid for one account per computer and licenses can be transferred between computers. To transfer a license, please deactivate it first via the *Licensing Status* dialogue and reactivate it on the new computer.

## 2. Term Definitions

**Merge Set:** A series of replicates of samples from a single or multiple data files collected with AN.Control, with identical temperature settings. Data within one Merge Set will be averaged and the standard deviation calculated. The software will additionally calculate and display error bands in the charts.

**Analysis:** A complete dataset consisting of any number of Merge Sets or single Andromeda samples from single or multiple data files, for comprehensive and systematic analysis and direct comparison. The analysis can be saved as an analysis file (.ana) at any time.

**Raw Data:** All data recorded by the Andromeda instrument: fluorescence traces of single wavelengths at 670 nm (RED) or 510 nm (BLUE). When exporting Raw Data, this also includes derived values such as first derivative and standard deviations for each data point.

### 3. Home Screen and General Layout

To perform a new analysis, start the AN.Stability Analysis software, which will show the analysis home screen. Click on *AN.Control Analysis* to create an analysis from data recorded with AN.Control software. Select the respective .anc file(s). The analysis will be saved in .ana format.

Alternatively, previous analysis files can be loaded by clicking *Browse*. Recently loaded files are listed chronologically on the right.

Use the *Save Analysis* button at any time to save modifications of the analysis file. An asterisk (\*) in the title bar indicates unsaved changes in the current analysis. Closing the software will trigger a dialogue box asking whether you want to save the changes.

All major functions of the AN.Stability Analysis software are organized in 4 different sections (see Figure 1):

1. Sidebar
2. Menu Bar
3. Data Table
4. Charts and Display Options

Additional buttons in the menu bar are:

5. Quick saving of the analysis file
6. Support

Alerts will be displayed on the top right of the main window.

The keyboard shortcut ctrl + z will undo any action, while ctrl + y will redo.

5. QUICK SAVING

6. SUPPORT

Andromeda - AN.Stability Analysis v1.1\*

Assign Reference Merge Sets Change Color Choose Key Parameters Edit Legend Labels

2. MENU BAR

1. SIDE BAR

3. DATA TABLE

General		Annotations			
Exclude	Thumbnail	Capillaries	Sample ID	Buffer	6His-p38
<input type="checkbox"/>		1, 2, 3,...	1	10 % E. coli Lysate i...	2000 nM
<input type="checkbox"/>		5, 6, 7,...	2	10 % E. coli Lysate i...	1000 nM
<input type="checkbox"/>		9, 10, 11,...	3	10 % E. coli Lysate i...	500 nM
<input type="checkbox"/>		13, 14, 15,...	4	10 % E. coli Lysate i...	250 nM
<input type="checkbox"/>		17, 18, 19,...	5	10 % E. coli Lysate i...	125 nM
<input type="checkbox"/>		21, 22, 23,...	6	10 % E. coli Lysate i...	62 nM
<input type="checkbox"/>		25, 26, 27,...	7	10 % E. coli Lysate i...	31 nM
<input type="checkbox"/>		29, 30, 31,...	8	10 % E. coli Lysate i...	16 nM
<input type="checkbox"/>		33, 34, 35,...	9	10 % E. coli Lysate i...	8 nM
<input type="checkbox"/>		37, 38, 39,...	10	10 % E. coli Lysate i...	4 nM
<input type="checkbox"/>		41, 42, 43,...	11	10 % E. coli Lysate i...	2 nM
<input type="checkbox"/>		45, 46, 47,...	12	10 % E. coli Lysate i...	0 nM

4. CHARTS AND DISPLAY OPTIONS

670 nm [counts · mm]

First Derivative

Temperature [°C]

Display Options X

Data Points

Smoothed Curve

Detectors

- 670 nm
- 510 nm

First Derivative

- Show First Derivative

Markers

- Show Onsets
- Show Inflection Points
- Show Fitted Tms

Reference

- Show Reference Profile

Regions of Interest

- Show Regions of Interest
- Show Fitted Curves

Chart Grid Lines

- Show Grid Lines

[IsVisible] = 'True'

Regions of Interest (0) Key Parameters (0)

Figure 1: General layout of AN.Stability Analysis.

## 4. Sidebar





Figure 2: Functionalities located in the sidebar.

### 4.1. Analysis Data

The *Analysis Data* menu allows adding, removing and filtering data files from AN.Control.

When creating a new analysis, enter an analysis name and optionally add comments, e.g. purpose of the analysis, assay conditions etc.

To add more data to the analysis use the *Add Files* function to browse folders and select single or multiple .anc files. Runs of the selected file(s) will appear in the list of loaded files in the Analysis Data window. Click on  to show only samples from the selected datafile or experiment. Click on  to remove single experiments or a whole datafile from the Analysis. Alternatively, clicking Remove Excluded Samples will delete single samples or Merge Sets from the Analysis after they have been marked as excluded in the data table.

**Note:** Files can also be added to the analysis directly from Windows Explorer by using drag-and-drop.

### 4.2. Global References

The *Global References* menu allows saving of a reference currently assigned in the Analysis. Saved global references can be reused later on any other data set by clicking Assign. References are saved locally on the PC's hard drive. To access them, open Windows Explorer and type %localappdata%\Nanotemper in the address bar. Reference files can be copied and moved to different PCs or users and reused there.

For more information on setting references, see 5.1.

### 4.3. Analysis Templates

*Analysis Templates* consist of Key Parameters and Regions of Interest and can be saved from the current data set. Reuse them later on any other data set. When an analysis template is applied to a data set:

- The selected Key Parameters are automatically plotted for all samples in the data set.
- Regions of Interest are applied and evaluated on the selected samples or on all samples in the data set, depending on whether the *Apply to all samples* checkbox is checked.

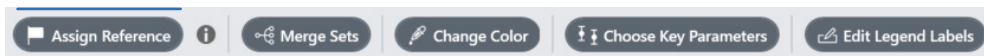
Analysis Templates are saved locally on the PC's hard drive. To access them, open Windows Explorer and type %localappdata%\Nanotemper in the address bar. Analysis template files can be copied and moved to different PCs or users and reused there.

### 4.4. Batch Export

Export selected samples or the complete Analysis Set. Please refer to chapter 8 for details on available options.

## 5. Menu Bar

### 5.1. Assign Reference

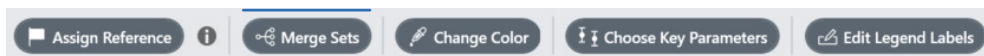


AN.Stability Analysis allows to set any sample in the current dataset as a reference. This can be either a Merge Set or a single capillary. Clicking the *Assign/Unassign Reference* button will assign/unassign the currently selected sample as reference. Only one reference can be used at a time.

The reference will be highlighted in the data table and its unfolding profile is shown in the charts in gray (if selected to be shown via the Display Options).

References can also be saved as Global Reference (see 4.2.) and reused later on any other dataset.

### 5.2. Merge Sets

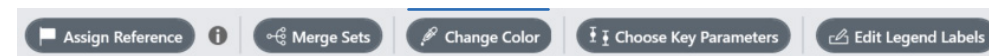


AN.Stability Analysis automatically detects and merges replicates by their annotation(s). By default, merging is and all available annotation columns are used to identify replicates. Merging is case-sensitive. Which annotation columns in the original data file are used for merging can be customized by checking or unchecking the respective checkbox in the *Merge Sets* menu. Unchecking *Enable merging of replicates* will undo all merging operations.

**Note:** Merging can alternatively be performed by dragging and dropping individual capillaries or Merge Sets in the data table.

**Note:** Samples acquired with different heating rates will not be merged, even if otherwise fully identical.

### 5.3. Change Color



The coloring scheme assigned and stored in the .anc files is imported into AN.Stability Analysis. Coloring can be changed and customized for individual capillaries, Merge Sets or the complete Analysis. Options are Single color, Gradient color, or Category color.

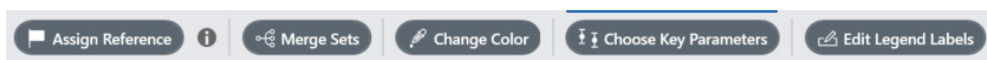
To color by gradients requires selecting multiple samples. The colors will directly translate into the curves displayed in the charts and Key Parameter plots. Color assignment will only apply to selected samples.

Multi-selection by using shift / ctrl + left click is possible.

The dialog box shows three main options: 'Single color', 'Gradient color', and 'Category color'. The 'Single color' option is expanded, showing 'Automatic', 'Theme Colors', 'Standard Colors', and a 'Color picker' icon. The 'Gradient color' and 'Category color' options are also expanded, showing a vertical gradient bar and a horizontal bar with colored segments, respectively. Three blue callout boxes provide instructions: the top one points to the 'Single color' section, the middle one points to the 'Gradient color' section, and the bottom one points to the 'Category color' section.

Figure 3: Coloring options.

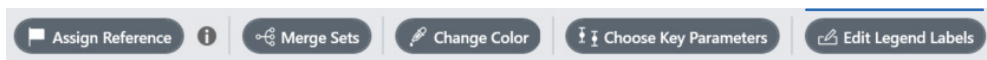
## 5.4. Choose Key Parameters



Individual output parameters can be plotted for the whole Analysis with the Key Parameter feature. Select from the list the parameters to be plotted by checking the respective checkboxes. The plot(s) will be displayed below the data table and contain all samples of the current Analysis, for which the parameter is available (see Figure 4). Capillaries or Merge Sets, for which the *Exclude* checkbox is checked in the data table, will not be shown in the Key Parameter plot. Each sample is depicted as data point and error bars indicate the standard deviation for average values from Merge Sets. Click on a data point to select it in the data table.

Key parameter plots can be exported to Excel™ (.xlsx) or as image (.png).

## 5.5. Edit Legend Labels



Define in the *Edit Legend Labels* menu, which annotations should be used in the legends of the charts; for exporting or to identify samples in the Key Parameter plot. Legend labels will by default be set to automatic mode, but you can switch to manual mode to select other label types.

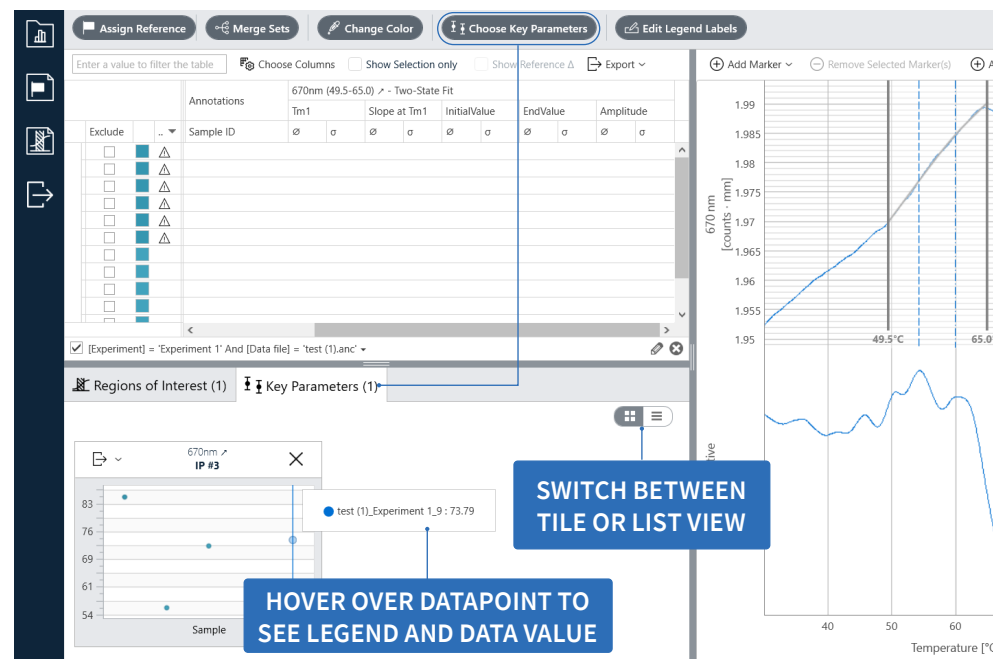


Figure 4: Additional functionalities in Key Parameter plots.

## 6. Data Table

### 6.1. Sample Organization and Merge Sets

Replicates will be automatically combined into Merge Sets upon loading data into AN.Stability Analysis. Merge Sets are shown as a tree structure in the data table (see Figure 5). For each Merge Set, average values and standard deviations are calculated automatically.

Merging behavior can be customized via the *Merge Sets* function in the menu bar (see 5.2.). Alternatively, samples can be moved between different Merge Sets by drag and drop.

Navigate between samples by using arrow keys up/down, and right/left to expand and collapse Merge Sets.


Single samples, replicates or whole Merge Sets can be excluded from the analysis by checking the *Exclude* checkbox (see Figure 5). It is possible to completely delete these samples from the Analysis via the Analysis Data tab located in the sidebar (see 4.1.)



Multi-selection of several rows by using shift / ctrl + left click is possible.

All available annotation columns will be loaded from the AN.Control file. Annotations can be manually changed by double-clicking into the respective cell. Content from single annotation cells may be copied to other annotation cells using copy-and-paste after selection. Changing an annotation for a Merge Set will change this annotation for replicates contained in the Merge Set. Additional annotation columns can be added by using *Choose Columns* (see following section).

### 6.2. Customizing Views

The data table includes separate groups of columns for general information on the experimental settings, annotations, and output parameters. The order of columns and column groups can be changed by dragging and dropping the respective header cell into a new position. Pin column groups to the left or right end of the table by right clicking on a group header cell and selecting *Left* or *Right* in the *Fixed Style* menu.


Sort values by clicking on the column header. The  next to the header indicates the sorting order (ascending, descending, or off). Alternatively, rightclicking on column header opens a menu with sorting options.

Hovering over a header cell makes  visible. Click it to open the filter menu and filter samples based on selected values. The  will stay visible in the header after a filter has been applied to a particular column (see Figure 5).

Which columns are shown can be customized via *Choose Columns* by checking/unchecking the respective checkbox in front of the column name or by clicking *Select All*. The list contains all columns available for the current analysis and only a subset will be shown by default. You can always return to this default by clicking *Reset to default*.

### 6.3. Output Parameters

Output parameter columns are grouped based on the data they originate from: 670 nm, 510 nm, Regions of Interest/Fits.

Arrows in the column headers indicate that the values were determined during a heating ramp .

Values for inflection points as well as manual markers are imported from the AN.Control file. They are sorted into respective columns based on temperature. Manual markers are shown in italics.

The data table can be exported in Excel™ format (.xlsx) or csv.

Figure 5: Functionalities in the data table.

The screenshot shows a data table with columns for 'Exclude', 'Thumbnail', 'Capillaries', 'Sample ID', and '510nm' (with sub-columns for IP #1 and IP #2). Callouts point to various features:

- SELECT TO EXCLUDE SAMPLE FROM ANALYSIS (AUTOMATICALLY CHECKED WHEN AIR BUBBLE WAS DETECTED)**: Points to the 'Exclude' checkbox.
- FILTER TABLE FOR ANNOTATIONS AND/OR VALUES**: Points to the search bar at the top.
- SELECT TO SWITCH FROM ABSOLUTE VALUES TO VALUES RELATIVE TO AN ASSIGNED REFERENCE**: Points to the 'Show Reference' checkbox.
- CLICK TO EXPAND OR COLLAPSE ALL**: Points to the expand/collapse icon.
- WARNING SIGNS INDICATE AN AIR BUBBLE WAS DETECTED**: Points to a warning triangle icon in the 'Thumbnail' column.
- MERGE SETS**: Points to the 'Merge Sets' checkbox.
- REPLICATES**: Points to the 'Replicates' checkbox.
- CLICK TO EXPAND OR COLLAPSE MERGE SET**: Points to the expand/collapse icon for a merge set.
- AVERAGE VALUES CALCULATED FROM INCOMPLETE DATA SETS ARE COLORED ORANGE**: Points to an orange value in the '510nm' column.

Exclude	Thumbnail	Capillaries	Sample ID	510nm
				IP #1
				σ
<input type="checkbox"/>			T07/B08 - 3	48.99
<input type="checkbox"/>			T07/B08 - 3	49.26
<input type="checkbox"/>			T07/B08 - 2	34.85 ±0.20
<input type="checkbox"/>			T07/B08 - 2	49.33 ±0.10
<input type="checkbox"/>			T07/B08 - 2	49.37
<input type="checkbox"/>			T07/B08 - 2	49.26
<input type="checkbox"/>			T07/B08 - 2	49.24
<input type="checkbox"/>			T07/B08 - 2	49.46
<input type="checkbox"/>			T07/B08 - 1	49.33 ±0.33

## 7. Charts and Display Options

All samples selected in the data table will be displayed in the charts panel. Multi-select samples by using shift or ctrl + left click.

For Merge Sets, the software will automatically calculate and display error bands.

Zoom into the graph using the scrolling function of the mouse wheel. Holding the mouse wheel also allows to move the graph horizontally. To reset the view, click *Reset Zoom*.

The calculated inflection points (IP) can be displayed in the charts by checking *Show Inflection Points* (see Figure 6).

Shown are also the unfolding profiles and, if selected, the respective first derivative of the curves. In the first derivative view, each local maximum or minimum corresponds to an Inflection Point. Inflection points can be manually added or removed by clicking the *Add/Remove Marker* buttons. Removing markers on the Merge Set will remove the respective value from all replicates within this set. Manual markers can only be added to individual replicates, but not to Merge Sets.

Inflection points can be added to the 670 nm and 510 nm plots. The manually annotated markers will be added in italics to the data table and sorted into the respective column based on their temperature value.

Any chart displayed in the charts panel can be exported by clicking the *Export* button . Options are to save it as an image (.png) or vector graphics (.pdf).

### 7.1. Regions of Interest

Data from AN.Control (670 nm or 510 nm) can additionally be fitted to a thermodynamic model.

To perform a fit, first select which type of fit (Two-state fit or Three-state fit) you want to use via the *Add Region of Interest* menu and then select

the respective region by clicking at the start and end point in the plot (see Figure 6). The calculation of fit parameters will then be carried out automatically.

Calculated values will be added as separate columns in the data table.

All defined Regions of Interest will appear in a separate panel below the data table next to the Key Parameters .

ROI can be saved as Analysis Templates (see 4.3.).

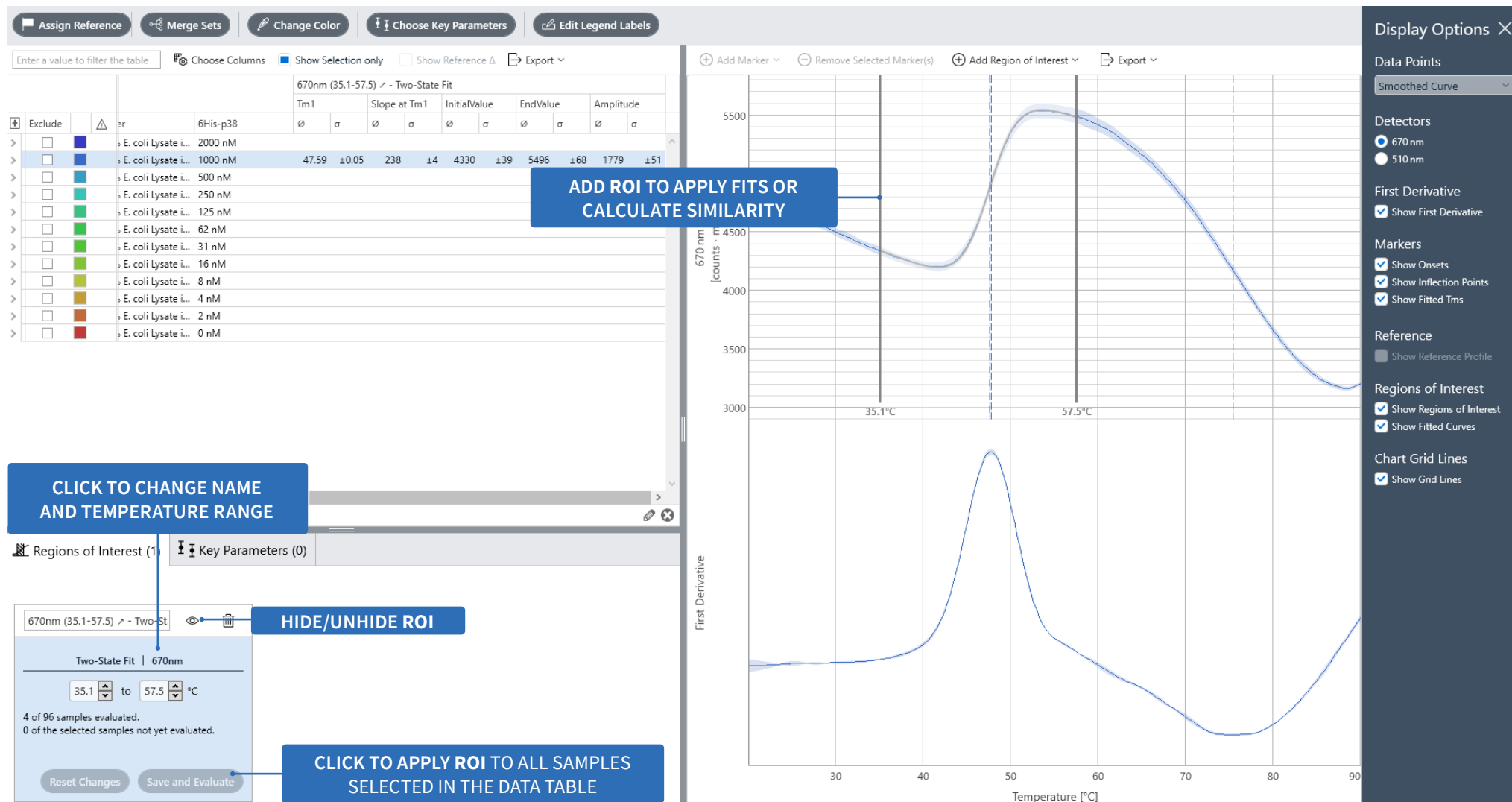


Figure 6: Display options in the charts panel and working with Regions of Interest (RoI).

## 8. Save and Data Export

Each measurement and analysis performed can be exported to be used in third-party software. Throughout this software manual, different export options are mentioned. This section aims to provide an overview regarding the available file formats.

Access the export menu(s) via the  button and select from the following options:

- The data table can be exported in Excel™ format (.xlsx) or as .csv
- Graphs can be exported as an image (.png) or vector graphics (.pdf)
- Key Parameter plots can be exported to Excel™ format (.xlsx) or as image (.png)

The *Edit Legend Labels* menu lets you define which annotations should be used in the legends of the charts (see 5.5.). These labels will be used for exporting or to identify samples in the Key Parameter plot.

Additionally, the full Analysis or selected parts can be exported via the *Batch Export* function in the sidebar (see 4.4.). This includes processed as well as Raw Data. Batch export settings can be saved as presets.

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