User Manual
PR.Stability Analysis

Content

NanoTemper® Technologies' Prometheus™ instruments detect changes in the fluorescence of the amino acid tryptophan (and fluorophores with equivalent spectroscopic properties) over a wide range of temperatures using nanoDSF™, an advanced Differential Scanning Fluorimetry technology. The instruments can be used to induce thermal unfolding of proteins and to determine thermal unfolding transition temperatures. Furthermore, the instruments are equipped to investigate chemical unfolding and the free energy of unfolding ΔG in an extraordinarily straightforward and fast manner.

The PR.Stability Analysis software is dedicated to analyzing thermal and chemical unfolding data acquired with PR.ThermControl and PR.ChemControl software on Prometheus 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, PR.Stability Analysis software can be installed on additional computers for convenient data analysis. The computers have to meet the following requirements:

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

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

1.2. Compatibility

Measurements collected with all versions of PR.ThermControl (previously called PR.Control) and PR.ChemControl software are compatible with PR.Stability Analysis.

Note: *PR.Stability Analysis is incompatible with PR.ThermControl-CFR.*

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 PR.ThermControl or PR.ChemControl, with identical temperature settings. Data within one Merge Set will be averaged and the standard deviation calculated. For data from PR.ThermControl, the software will additionally calculate and display error bands in the charts.

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

Raw Data: All data recorded by the Prometheus instrument: fluorescence traces of single wavelengths and ratio, scattering signal. 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 PR.Stability Analysis software, which will show the analysis home screen. Click on *PR.ThermControl Analysis* or *PR.ChemControl Analysis* to create an analysis from thermal or chemical unfolding data, respectively. Select the respective run file(s) in .prc or .prcc format. The analysis will be saved in .pra 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.

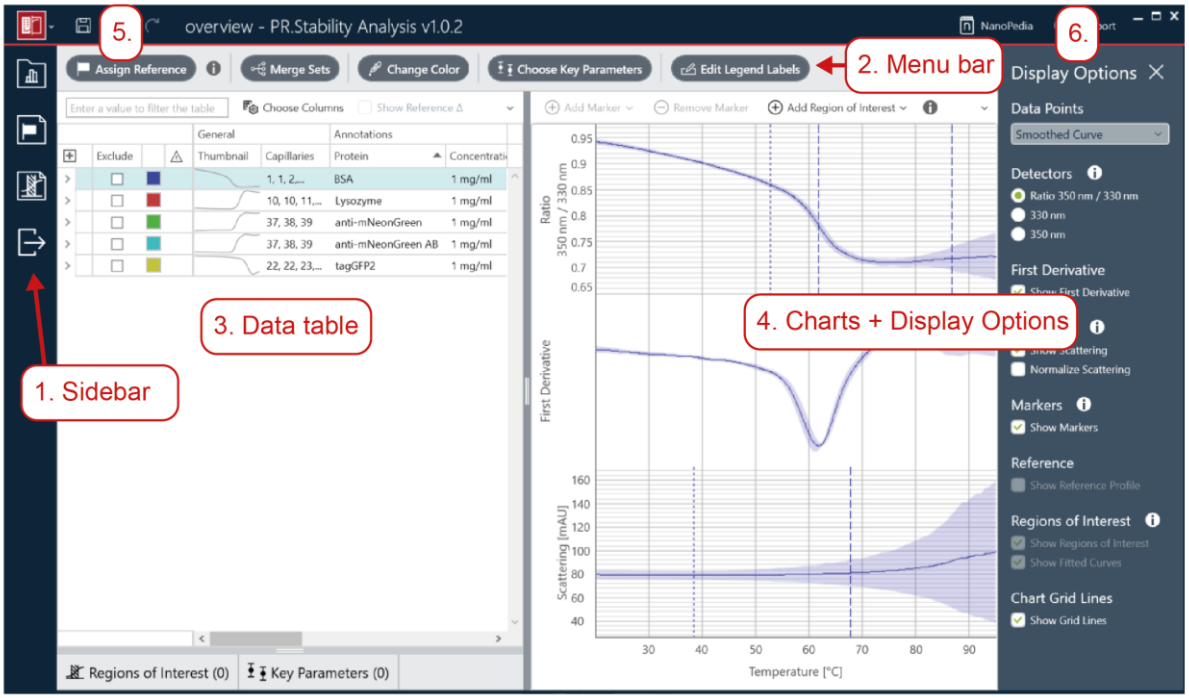


Figure 1: General layout of PR.Stability Analysis.

All major functions of the PR.Stability Analysis software are organized in 4 different sections:

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. Access to NanoPedia

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

Context-related supporting information, such as term definitions, can be found when hovering over items and in the NanoPedia.

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

4. Sidebar

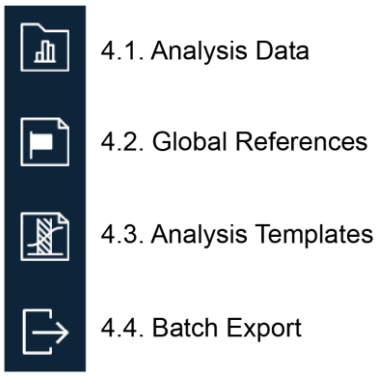


Figure 2: Functionalities located in the sidebar.

4.1. Analysis Data

The *Analysis Data* menu allows adding, removing and filtering data files from PR.ThermControl and PR.ChemControl.

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 .prc or .prcc 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. Click on  to remove single experiments or the whole datafile from the Analysis Set. Alternatively, clicking *Remove Excluded Samples* will delete single samples or Merge Sets from the Analysis Set 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.*

If you are using PR.ThermControl and PR.ChemControl with older versions of algorithms, you can re-analyze the Analysis Set with the newest algorithms by clicking on *Update Algorithms*. This applies to PR.ThermControl version 2.1 and older, as well as PR.ChemControl version 1.4 and older.

4.2. Global References

The *Global References* menu allows saving of a reference currently assigned in the Analysis Set. 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.

Global references are only available for PR.ThermControl analysis.

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.

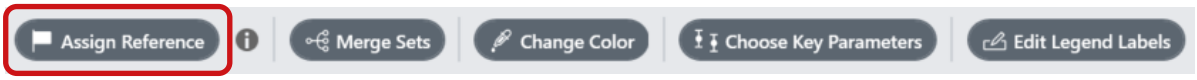
Analysis templates are only available for PR.ThermControl analysis.

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

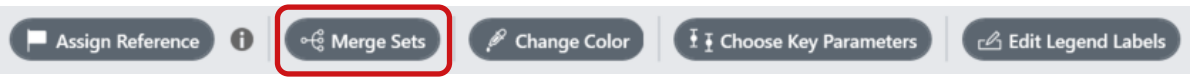


PR.Stability Analysis allows to set any sample in the current dataset as a reference. This can be either a Merge Set or a single capillary. Clicking the *Assign/Unassign Reference* button will assign/unassign the currently selected sample as reference. Only one reference can be used at a time.

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



PR.Stability Analysis automatically detects and merges replicates by their annotation(s). By default, merging is enabled 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 .prc or .prcc files is imported into PR.Stability Analysis. Coloring can be changed and customized for individual capillaries, Merge Sets or the complete Analysis Set. 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.

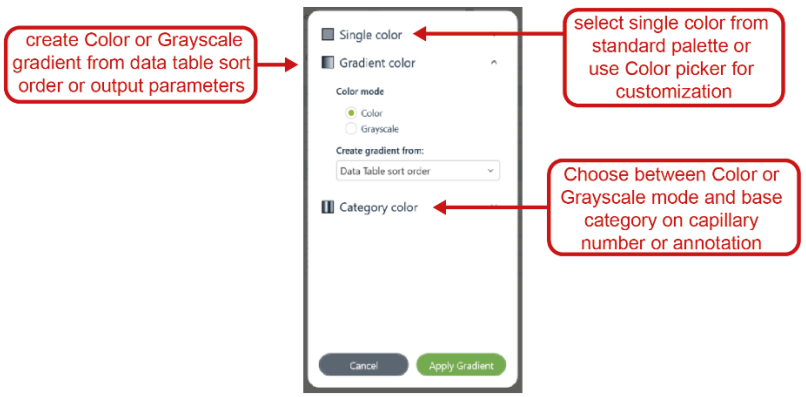
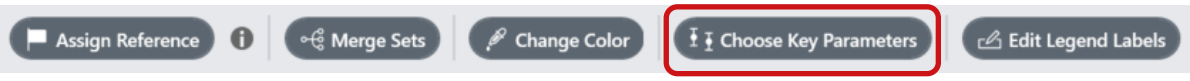


Figure 3: Coloring options.

5.4. Choose Key Parameters



Individual output parameters can be plotted for the whole Analysis Set 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 Set, for which the parameter is available (see Fig. 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).

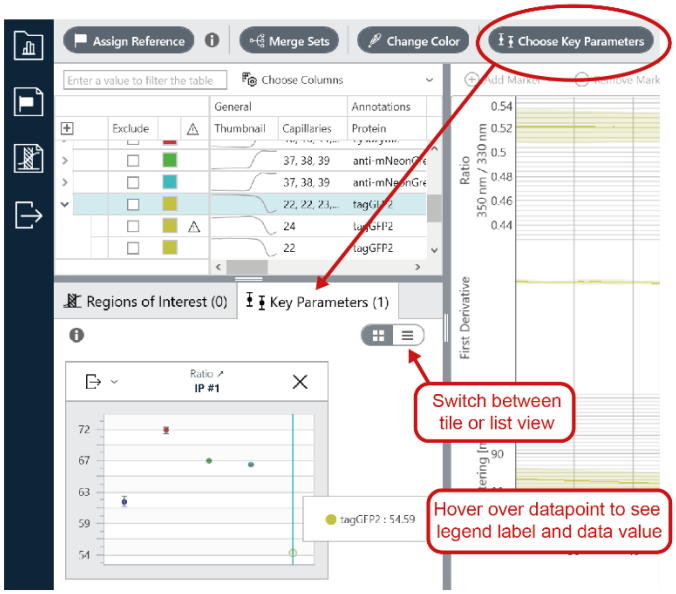


Figure 4: Additional functionalities in Key Parameter plots.

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.

6. Data Table

6.1. Sample Organization and Merge Sets

Replicates will be automatically combined into Merge Sets upon loading data into PR.Stability Analysis. Merge Sets are shown as a tree structure in the data table (see Fig. 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 Fig. 5). It is possible to completely delete these samples from the Analysis Set 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 PR.ThermControl or PR.ChemControl 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 header cell and selecting of *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 Fig. 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: Ratio, single wavelengths, Scattering, Regions of Interest/Fits.

Arrows in the column headers indicate whether the values were determined during a heating ramp ↗ or a cooling ramp ↘. Parameter definitions can be found in NanoPedia.

Values for inflection points, onset of unfolding and onset of aggregation, as well as manual markers are imported from the PR.ThermControl file. They are sorted into respective columns based on temperature. Manual markers are shown in italics.

The output parameters ΔG and c_{50} from PR.ChemControl files will also be imported into PR.Stability Analysis.

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

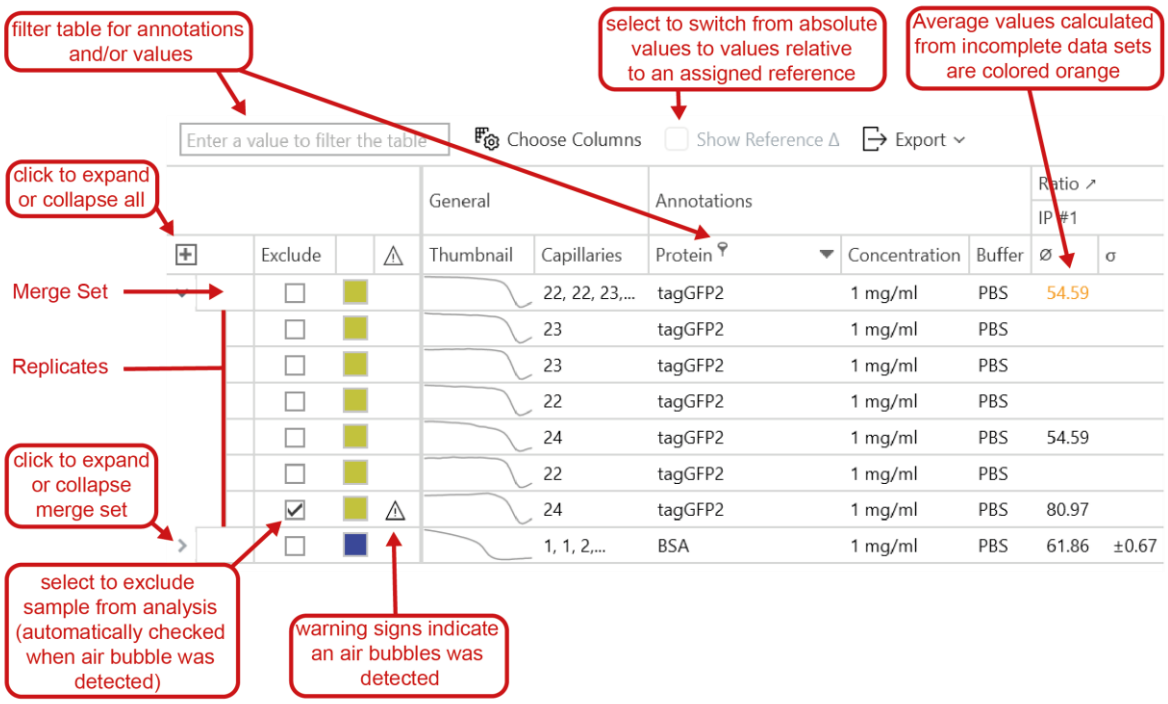


Figure 5: Functionalities in the data table.

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. If the Prometheus instrument is equipped with Aggregation Optics, the Scattering signal is also displayed for each individual sample or Merge Set.

For data from PR.ThermControl, the software will additionally 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) and unfolding onset temperatures can be displayed in the charts by checking *Show Markers* (see Fig. 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 a transition temperature. Inflection points and unfolding onsets 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. Onsets can be added to the Ratio and Scattering plot; Inflection points to the Ratio and single wavelengths (330 nm and 350 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.

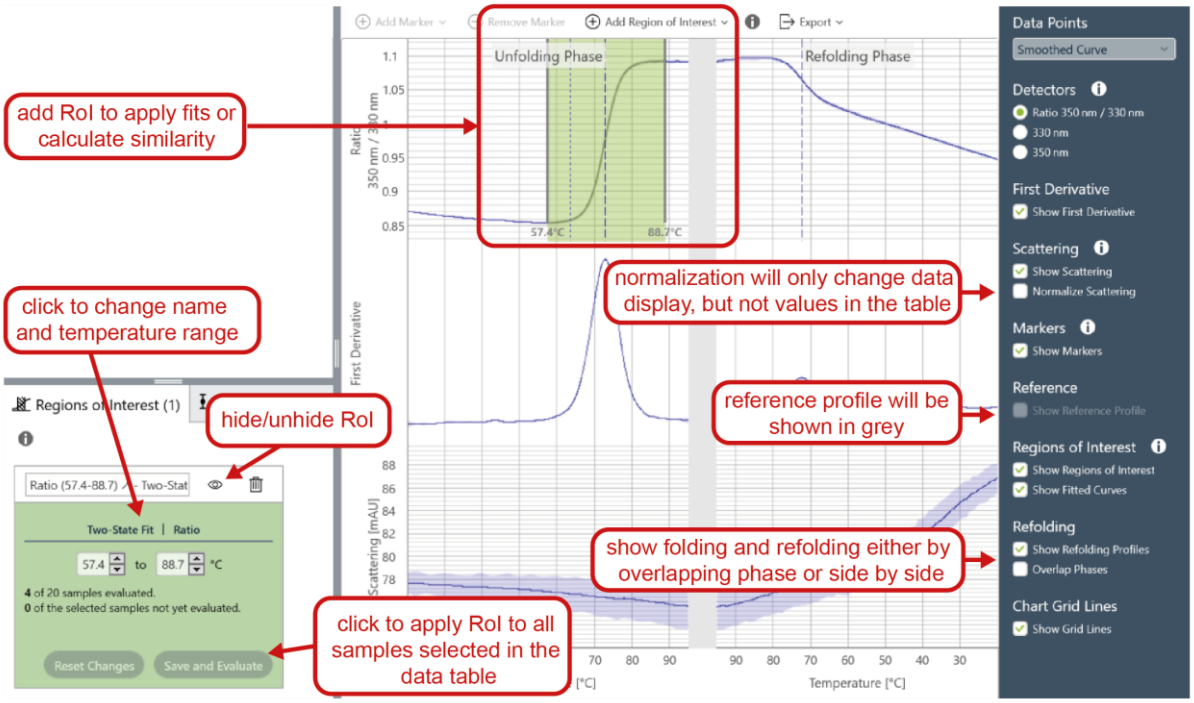


Figure 6: Display options in the charts panel and working with Regions of Interest (RoI).

Data from PR.ChemControl is always fitted to a thermodynamic model instead of using derivatives to calculate transition midpoints. Therefore, these plots do not contain markers. The fitting model will be loaded from the .prcc file(s). To change between Two-state and Three-state fitting, select the respective model from the dropdown menu above the charts. Click on outlier data points in the plot to exclude them from the analysis. Excluded data points will be shown in gray.

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 PR.ThermControl (Ratio or single wavelengths) 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 Fig. 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 (see Fig. 6).

Regions of Interest (RoI) can also be applied to recalculate the similarity to an assigned reference for a specific part of the ratio profile.

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

Note: *Data from PR.ChemControl is always fitted to a thermodynamic model as a whole, without the application of RoIs.*

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 Set 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.

9. NanoPedia

NanoPedia contains term and parameter definitions for PR.Stability Analysis as well as background information on Prometheus systems, data interpretation, and protein stability analysis in general. It is saved locally in HTML format on the PC's hard drive upon installation of the software. To access NanoPedia, click on  in the menu bar. Alternatively, click on one of the info buttons  that can be found next to various items in the software and will link to context-related articles.

NanoPedia will open in the PC’s standard browser and is compatible with all browser types. Internet access is not required to access NanoPedia. Clicking on one of the buttons within PR.Stability Analysis will open the respective article or the NanoPedia main page in a separate tab. Use the browser’s printing function to export the selected article into .pdf format.

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